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News

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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTY-NINTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY PALO ALTO, CALIFORNIA, AUGUST 26, 27, 28, 1957

Ohio W-R Seven, a new disease-resistant globe-type glasshouse tomato variety. ALEXANDER, LEONARD J. Ohio W-R Seven is being introduced to growers of glasshouse tomatoes as a new disease-resistant variety. This variety carries the dominant I gene for resistance to race 1 of *Fusarium oxysporum* f. *lycopersici*. It is free of the fruit blemish, pox. Ohio W-R Seven is also highly resistant to the blotchy ripening disease of tomato, but under extreme conditions an occasional blotchy ripened fruit has been observed. The fruits of Ohio W-R Seven are globe shaped, thick walled, meaty, firm, and pink. Cracking is of the radial type. Its maturity is midseason. Production is comparable to that of Ohio W-R Globe. In 7 spring crops, Ohio W-R Globe produced 14.6 lb. per plant whereas Ohio W-R Seven produced 14.3. In 8 fall crops, the former produced 8.1 lb. per plant whereas the latter produced 8.0. These differences may or may not be significant. Fruit size is approximately 6 oz.

Tobacco mosaic virus inhibition by rice extracts. ALLEN, THOMAS C., JR., AND ROBERT P. KAHN. When primary leaves of Pinto bean were inoculated with a tobacco mosaic virus (TMV) inoculum diluted with an equal volume of water, approximately 150 lesions were produced per half leaf. No lesions developed, however, when comparable leaves were inoculated with the inoculum diluted with an equal volume of an extract from rice leaves. This suggested the presence of an inhibitor or inhibitors in the rice juice. An inhibitor was extracted from rice flowers, polish, roots, kernels, and culms and from heat-dried, frozen, or fresh leaves. Lesion inhibition was demonstrated on bean leaves dipped in a rice polish suspension and immediately rinsed; inhibition occurred whether the leaves were dipped before or after inoculation with TMV. Application of the suspension to the lower leaf-surface also reduced the lesion number. The inhibitor extracted from leaves and from roots was highly active in dilutions up to 1:200; that extracted from rice polish was active at dilutions up to 1:6000. Apparently the inhibitor is not heat labile.

The mutagenic and injurious effect of filipin on Helminthosporium sativum. ALLISON, PATRICIA, AND J. J. CHRISTENSEN. The frequency of mutation in *Helminthosporium sativum* was greatly increased by the use of filipin. Induced mutants differed in various degrees from their parent in many physiological and morphological characters. At certain concentrations, filipin inhibited or retarded germination of spores and growth of the colonies. Filipin induced the formation of tumor-like bodies either from within the spore or from within the young sporeling. These aggregates resembled galls or tumors produced by bacteria or viruses in higher plants and consisted of hundreds of cells of diverse sizes and shapes. The overgrowth frequently was associated with rupturing of the epispores and cell walls, extrusion of the protoplasm, and abnormal thickening of the walls; cells sometimes formed endogenously in catenulate manner. Sometimes normal hyphae emerged from these overgrowths, depending on time and concentration of filipin. Some overgrowths without hyphae have survived for 7 weeks, yielding apparently normal mycelial colonies when transferred to filipin-free medium.

Use of antibiotics and other chemicals for control of common and fuscous blights of beans. ANDERSON, AXEL L. Navy pea beans in 0.01-acre plots were sprayed weekly over a 7-week period, with various antibiotics and other chemicals, beginning when the plants were in the 1-trifoliate-leaf stage. Control data were based on natural field infection with *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans*. The disulfide, sodium, zinc, and copper derivatives of 2-pyridinethiol,1 oxide were tested alone and in combination with streptomycin sulfate. Albomycin, streptomycin sulfate, and various streptomycin formulations (Agrimycin 100 and 500, HL 881, HL 908, and Phytomycin) also were tested. In the pyridinethiol series, only the copper salt at 500 ppm gave control. The compounds containing streptomycin sulfate were more effective than the copper salt of pyridinethiol in preventing infection. When combined with 200 ppm of streptomycin sulfate, the copper salt of pyridinethiol stimulated plant growth, improved the color, and increased yield. Addition of 1% glycerol increased the bactericidal activity of sprays containing 200 ppm streptomycin sulfate but not that of sprays containing 400 ppm. Nontreated controls averaged 6000 infected trifoliate leaflets and 10.6 g of infected beans per 0.01-acre plot, compared with 1.5 infected trifoliate leaves and 0.7 g of infected beans in the plots sprayed with 400 ppm streptomycin sulfate.

Seed transmission of three viruses in cowpea. ANDERSON, C. W. Two strains of cowpea mosaic virus and 1 of cucumber mosaic virus were studied in 4 cowpea varieties. The relationship of the cowpea mosaic virus strains was based on knowledge of reactions on indicator plants and of immunological relationships. Amounts of seed transmission were 0–1% and 0–55% for the 2 strains of cowpea mosaic virus, 4–28% for cucumber mosaic virus, and 0% for the controls. All 3 viruses were most readily seedborne in White Acre Pea. Only cucumber mosaic virus was seedborne in Yardlong Bean. Seed transmission of cucumber mosaic virus was confirmed by means of sap transmission to pepper plants.

Effects of X rays on the wheat stem rust fungus, Puccinia graminis var. tritici. ANDERSON, ROGER V. Dormant or germinating urediospores and mycelia of race 48A were X rayed with dosages from 15,000 to 45,000 roentgens(r). No lethal effects followed irradiation of dormant spores, subsequent germination equaling that of nonirradiated spores. Germ tubes of germinating spores survived 20,000 r, but half of them died following treatment with 30,000 r. Ability to infect wheat was reduced when dormant or germinating spores received 30,000 or 45,000 r. There were lethal effects on mycelia of the rust fungus in Khapli leaves X rayed with 25,000 r at intervals after seedlings were inoculated. With X rays 24, 48, or 72 hours after inoculation, only 1–2% as many uredia formed as in nonirradiated controls within 2 weeks after inoculation. After an additional 2 weeks, uredia increased by 1–5%, and 1-2% flecks appeared. With X rays 96 hours after inoculation, 64% of the infections (compared with nonirradiated controls) recovered sufficiently to produce flecks but no uredia within the 2 weeks. Four weeks after inoculation, 5% of the infections bore uredia and 64% were flecks. X rays killed some of the rust fungus immediately, delayed rust development, and often inhibited sporulation. Parasitic phases of the fungus were more sensitive to X rays than were the dormant or germinating spores.

Cercospora leaf spot of Photinia serrulata. ANZALONE, L., JR., AND A. G. PLAKIDAS. Nurserymen in Louisiana have had difficulty for several years in growing salable plants of *Photinia serrulata*, mainly because of a disease caused by an apparently undescribed species of *Cercospora*. This organism attacks the foliage, causing numerous necrotic spots and severe defoliation. The fungus has been isolated repeatedly in pure culture from tissue plantings and grows well on potato-dextrose and oatmeal agars. Two species of *Cersospora* have been described on Photinia: *Cercospora heteromeles* Harkn. (on *P. arbutifolia*) and *C. eribotryae* (Enjoji) Saw. (on *P. glabra*). A third (undescribed) species (on *P. serrulata*) was reported in the "Index of Plant Diseases in the United States." The Louisiana Cercospora

is considered distinct from *C. heteromeles* on the basis of morphological differences and from *C. eriobotryae* on both morphological and hostal differences. The disease was reproduced on *P. serrulata* by inoculations with pure cultures (shredded mycelium) and with conidia from lesions on leaves of *P. serrulata*. Parallel inoculations of *P. glabra* and *Eriobotryae japonica* failed to produce the disease. Conidiophores of the Louisiana fungus are produced amphigenously on a globular stroma, are pale to olivaceous brown, and measure 2.6–3.9 × 10–43μ. The conidia are obclavate, hyaline, straight to mildly curved, and 1.9–2.8 × 29.9–99.3μ. The Louisiana Cercospora is designated *C. photinia-serrulatae* sp. n.

The height of invasion of two pathogens in carnation stems. BAKER, RALPH. During attempts to obtain pathogen-free carnations by means of the cultured-cutting technique, the wilt pathogen, *Fusarium oxysporum* f. *dianthi*, was occasionally isolated. The stem rot pathogen, *F. roseum* f. *cerealis*, was isolated only once during a 4-year period. To ascertain the extent of invasion of these pathogens in stems of carnation, stem segments from the lower 18 in. of stems of plants with symptoms of Fusarium wilt and Fusarium stem rot were cultured. *F. oxysporum* frequently was found in stems 18 in. above the base. *F. roseum* was occasionally isolated 6–8 in. above the base but usually no higher than 4 in. In a controlled experiment, plants were inoculated with the 2 pathogens and at intervals over a 12-month period platings were made from test plants at points up to 24 in. above the base of the stem. *F. oxysporum* was found at 18 in. in 1 instance but usually not more than 4–6 in. above the base of the stem. The maximum height at which *F. roseum* was isolated was 2 in. Cuttings taken from "mother" plants infected with *F. roseum* were cut into 2-mm sections, and these were cultured. The organism was not recovered from the cuttings. These results indicated that *F. roseum*, in contrast to *F. oxysporum*, is not likely to be carried in carnation cuttings.

A stage in the pathology of tobacco cells infected with tobacco mosaic virus. BALD, J. G. The cytological appearance of epidermal cells from healthy tobacco leaves was compared with that of cells in an early stage of infection before virus inclusions had formed. The methods of fixation and of staining maintained the color and form of chloroplasts and preserved mitochondria and spherosomes. Plastids were interpreted as complex structures containing an organiser, probably the primary granum in proplastids described by Strugger. A protein-containing spheroid or crystal, which was external to the lamellar portion containing the chlorophyll-bearing grana, was considered evidence of the plastid's capacity to form protein. Spheroids or crystals also occurred in modified proplastids. In infected mature cells, the cytoplasm increased, large trabeculae crossed the cell, and the enlarged nuclei had abnormal plastids on their surfaces. Plastids had prominent vacuoles, and the perimeters of the vacuoles or the whole plastids stained deeply. The whole cell was involved in pathological activity; association of the nucleus, center of nucleic acid synthesis, with plastids synthesizing protein suggested a possible dual origin for virus nucleoprotein.

Reassessment of virus host-range studies. BALD, J. G., AND T. W. TINSLEY. Plant species susceptible and insusceptible to certain viruses were arranged according to Hutchinson's classification of the Angiosperms. Holmes' claim that some groups of families were relatively susceptible and others insusceptible to tobacco mosaic virus (TMV) and to severe etch virus (SEV) was confirmed. Many of the same groups of families were similarly susceptible or insusceptible to the 5 viruses studied by Price. *Solanaceae* and *Scrophulariaceae* were very susceptible to many viruses. Below family level, the tribe *Solanae* contained more species with resistant or tolerant (not hypersensitive) reactions to TMV and SEV than did other tribes of *Solanaceae*. In *Nicotiana*, this resistance was confined to sections stated by Goodspeed to have dominantly Cestroid ancestry; it did not occur in sections with dominantly Petunioid ancestry. The irregular distribution of hosts susceptible to many viruses seem due to the combined effects of 1) the nature of the virus, 2) its previous adaptation to certain host species, and 3) the extent to which factors for resistance, either general or in some degree specific to the virus, are distributed among related families or groups of families and among taxa at lower levels.

The use of detached Ribes and pine leaves in studies with Cronartium ribicola. BEGA, ROBERT V. Detached leaves of both Ribes and pine species are being used in studies of the effect of microclimate on the fungus *Cronartium ribicola* and on the disease caused by it. Either single leaves or fascicles of needles or small branches bearing several fascicles are used. Ribes leaves have been kept alive up to 45 days in Petri dishes containing moist sand covered by blotter paper through which the petioles of the leaves are inserted. When tests are carried out in dark incubators, the sand is kept moist with a 10% sucrose solution. In this manner, leaves have been kept alive, and infection on them followed, for 30 days. To avoid the continuous moisture that occurs on leaves in closed Petri dishes, L-shaped tubes have been designed. Either a petiole or small branch is inserted into the horizontal end, and water, nutrient solution, or 10% sucrose solution is supplied from the top of the tube. Ribes leaves have remained alive up to 60 days in this way. With *Pinus* spp. the L-shaped tubes have proved more satisfactory than Petri dishes for small twigs containing several fascicles of needles. Twigs of *Pinus lambertiana* are still living after 6 months. For single fascicles of needles, 2 mm of the end of the fascicle is inserted into water agar in small glass vials. These needles are still living after 6 months.

Resistance to wheat streak mosaic virus in foreign and domestic wheats and various wheat crosses. BELLINGHAM, ROSCOE C., HURLEY FELLOWS, AND WEBSTER H. SILL, JR. During 7 years, 2477 foreign winter wheat introductions, 1965 foreign and domestic spring wheats, 99 domestic winter wheats, and many crosses with domestic and foreign varieties (including rye and Agropyron) have been tested for resistance to wheat streak mosaic virus. Field inoculations were by means of an abrasive spray at 25 lb./sq. in. Three standard nurseries were employed as follows: 1) elimination—to eliminate very susceptible varieties, 2) observation—a replicated nursery for possible resistant entries, and 3) yield—for final analysis of best entries. Reactions varied from extreme systemic susceptibility in most wheats and many crosses to resistance in a few wheat × Agropyron and wheat × rye crosses of doubtful agronomic value. Some tolerance, which varied greatly with environment, time of inoculation, and other factors, was present in a few domestic winter wheats (Bison, Kiowa, Triumph, Westar, Concho, Stafford, BlueJacket, some Apache crosses) and in 11 foreign varieties. In yield trials, minimum losses in these better varieties varied from 7 to 20% or more in different years. Approximately 2400 plant selections in several varieties were made to improve tolerance, all without success.

Infection studies with the southwestern cotton rust pathogen, Puccinia stakmanii. BERKENKAMP, BILL B., AND R. B. STREETS. *Puccinia stakmanii* Presley is a macrocyclic rust fungus, the pycnial and aecial stages of which attack cotton (*Gossypium* spp.); the uredinial and telial stages attack several species of grama grass (*Bouteloua* spp.). Teliospores were found to germinate only after being thoroughly wet or exposed to humidities of 100% at 20° C. At room temperature they begin germination in 7 hours. In the field, pycnia appear in 4 days and aecia in 14 days after rain. In greenhouse tests it was found that high humidities are not required for more than 24 hours for infection to occur. In similar tests, wet leaves seemed less susceptible than dry leaves under high humidity. Natural occurrence is very erratic. One experimental field had very slight infections in early July (2–10 sori per plant), a very severe infection

(50–200 sori per leaf) following 2 cloudy days with slight showers (July 30–31), and almost no infection during August and September. A parasite attacking the aecia of this rust fungus has been tentatively identified as *Tuberculina persicina*.

Studies of the physiology of the fungus Dothidella ulei. Blazquez, Carlos H., and John H. Owen. The growth of the fungus *Dothidella ulei* P. Henn. was studied *in vitro* and on Hevea rubber tree foliage growing under greenhouse conditions. Severe outbreaks of the disease in the greenhouse occurred only during long periods of high humidity, with accompanying temperatures ranging from 21°–32°C. On media prepared from leaf extract of *Hevea brasiliensis*, the fungus made excellent growth and produced an abundance of conidia. Maximum germination of conidia occurred at 24°–28°C and pH 7–8. Small concentrations of nutrient extracts were needed for growth of the fungus in agar media. Quebrachitol (2-monomethyl ether of *i*-inositol), which is present in Hevea rubber latex in concentrations of 0.5–2.0%, favored fungus growth when used at 100–200 mg per liter in all media tested. Combinations of *i*-inositol and nicotinic acid with glutamine and glycine, respectively, when added to a basic semisynthetic medium, resulted in good growth of the fungus. Growth *in vitro* did not appear to affect the pathogenicity of the fungus.

Cultural races of Mycosphaerella fragariae. Bolton, A. T., and H. N. Racicot. Many strawberry seedlings that were resistant to infection when repeatedly inoculated in the greenhouse with a conidium suspension from mixed cultures of *Mycosphaerella fragariae* (Tul.) Lindau proved susceptible in the field. This suggested that strains of the fungus more pathogenic than those used in the tests were present in nature. Isolations were made from 18 commercial strawberry varieties from different areas and from wild strawberries growing in uninhabited areas. Cultures of the organisms were obtained from Nova Scotia, Louisiana, and France. In all, 32 types of culture were obtained; these differed in colony color, pigment production, rate of growth, texture of stroma, and topography of colony surface. Each commercial strawberry variety was found to give rise to a distinct cultural type. Wild strawberries (*Fragaria virginiana*) yielded a number of cultural types. Microscopic examination revealed no great morphological differences. Variations or mutations of the fungus in culture were observed, quite frequently in some races and infrequently in others. Some variations occurred when a culture from 1 variety was inoculated onto and recovered from another variety a number of times. The cultural races of the fungus differed to some extent in pathogenicity.

Estimation of sedimentation constants of plant viruses by density-gradient centrifugation. Brakke, Myron K. The ratio of the sedimentation rate of a given plant virus to that of tobacco mosaic virus may be determined by centrifuging the 2 viruses at the same time through identical density-gradient columns. The ratio of the sedimentation rates is equal to the reciprocal of the ratio of the times required for the 2 viruses to sediment a given distance. Extrapolation of the ratios of the sedimentation rates at different densities to the density of water gives the ratio of the sedimentation constants of the 2 viruses. Determined in this way, the ratio of the sedimentation constant of southern bean mosaic virus to that of tobacco mosaic virus was 0.605; the value obtained from sedimentation constants in the literature is 0.615. The sedimentation constant of wheat streak mosaic virus was estimated to be 161±6 s (Svedberg units); that of barley stripe mosaic virus, 181±6 s; and that of brome mosaic virus, 77±6 s. Only 0.01–0.1 mg of virus is needed for a determination; it need not be highly purified, since the virus zone is readily identified by infectivity assay. With the above viruses and many others, crude extracts from diseased plants yield a satisfactory zone if the normal plant proteins are first partially denatured by heating, freezing, or chloroform-butanol treatment.

The production of new races of Puccinia graminis var. tritici by hyphal fusion on wheat. Bridgmon, G. H. Urediospores of "color-marked" races of *Puccinia graminis* var. *tritici* were mixed and injected hypodermically in aqueous suspension into the boots of wheat plants. When race 121, with gray-brown urediospores, was mixed with either race 9 or 11, both with orange spores, the resulting infection yielded 2 undescribed red races and 4 subraces. In general, each new isolate approached the pathogenicity of one of the parent races. Some of these isolates were more virulent than either of the parent races on certain varieties of wheat and less virulent on others. From a mixture of orange race 11 and gray-brown race 121, 2 new races and 3 subraces were isolated that were more pathogenic than race 11 or 121 on Bowie. From gray-brown race 121 mixed with orange race 9, a race 15 was isolated that was more pathogenic on Reliance than was either parent race. Most of these isolates were obtained more than once from successive mixtures. This is additional evidence that new races of the stem rust fungus may arise by hyphal fusion on the cereal host.

Mosaics of Italian ryegrass and orchard grass in western Washington. Bruehl, G. W., H. Toko, and H. H. McKinney. A mosaic of *Lolium multiflorum* Lam. was found in 4 counties west of the Cascade Mountains. The casual virus was transmitted with difficulty by use of Carborundum powder and expressed juice to *Lolium multiflorum*, *L. perenne* L., *L. remotum* Schrank., *L. temulentum* L., *Dactylis glomerata* L., *Festuca elatior* L., varieties of cultivated oats, *Avena fatua* L., and Michigan Amber wheat. Symptoms ranged from strong and persistent to faint and transient on these hosts. Faint and sometimes transient symptoms developed on *Bromus commutatus* Schrank., *B. mollis*, L., *B. racemosus* L., *B. secalinus* L., *B. tectorum* L., and *Hordeum leporinum* Link. A mosaic of orchard grass (*Dactylis glomerata*) was found in 4 counties of the same region. Inoculation trials so far have shown no differences between the viruses from ryegrass and orchard grass. Transmission trials with 3 aphids (*Macrosiphum granarium* Kirby, *Myzus persicae* Sulz. and *Rhopalosiphum fitchii* Sand.) have so far been negative.

Ultraviolet- and X-ray-induced mutation in Phytophthora cactorum. Buddenhagen, Ivan W. Mutants were induced by irradiating uninucleate zoospores of *P. cactorum* with 2250–6750 r of X rays and with low ultraviolet (UV) dosages, mainly 2537 Å from a 15-w germicidal lamp. Of surviving zoospores, a 30% mutation rate was obtained with UV of 15 seconds at 52 cm; a 50% maximum was obtained with a 110-second exposure. UV was superior to X rays as a mutagenic agent. Mutants in culture differed in size and morphology of colonies, in sporulation, in size, shape, and papillation of sporangia, and in size of oospores. Hyphae were changed from "smooth" wild type to "knobby," "clubby," or "spherical swelling" types. Two mutants produced nonpapillate sporangia resembling those of *P. hibernalis* and spherical swellings resembling those of *P. cinnamomi*. Differential growth on minimal medium, with or without vitamins or amino acids, was obtained among the mutants. No replaceable biochemical blocks were found in 300 mutants tested, but some did not grow on minimal medium; several mutants produced greater dry weights than did the wild type. Pathogenicity to peach trees, as measured by canker length, varied among the mutants from none to that of the wild type. Some mutants differed sufficiently to make their identification as *P. cactorum* impossible without previous knowledge of their origin. The differences among the mutants demonstrated the organism's potential for variation and the limitations of "species" in *Phytophthora*.

Chemical compositions of Bordeaux mixtures. Burchfield, H. P., Beatrice S. Magdoff, and Joan Schechtman. Freshly prepared Bordeaux mixture is an amorphous hydrogel that becomes crystalline on ageing. The crystalline phase consists of a copper-containing component, calcium

sulfate dihydrate, and free lime; the amount of the latter depends on the quantity used in the formulation. Tribasic copper sulfate ($Cu_4SO_4(OH)_6$) is formed when the minimum amount of lime required to precipitate all of the copper ion is used. When the amount of lime is increased, calcium and hydroxyl ions enter the crystal lattice so that a series of compounds with the general formula $Cu_4Ca_nSO_4(OH)_{6+2n}$ is formed where n is a number from 1 to 6, inclusive. Bordeaux mixtures having compositions of 10-6-100 to 10-10-100, inclusive, form sphaerocrystals that have similar X-ray diffraction patterns even though they contain different amounts of calcium. On prolonged ageing, the copper fraction of 10-6-100 Bordeaux is converted to $Cu_3Ca_2SO_4(OH)_6$. The copper fraction of 10-10-100 Bordeaux is changed to $CuCa_2SO_4(OH)_4$ at increased temperatures.

Control of club root of cauliflower. CAMPBELL, LEO. In experiments on the control of club root, fungicides were 1) added to the transplanting water and 2) mixed into the soil in strips. In the first method, each of 5 fungicides was used at the rate of 2.25 lb. active ingredient to 100 gal. of water; 300 ml of each preparation was used per plant. In comparison with untreated checks, zineb reduced club root development 90.3% and increased the yield of tops 360%, whereas Terraclor (75% pentachloronitrobenzene) reduced disease development 91.1% and increased yield 257.0%. Each of the other fungicides gave excellent control of the disease but did not increase yields significantly. In the second method, Terraclor was applied to the soil surface in strips from 2 to 12 in. wide and rototilled from 2 to 6 in. deep. Best results were from strips 8 in. wide and 4 in. deep, with Terrachlor applied at the rate of about 35 lb. per acre. Here club root development was reduced 83.6% and the yield of tops increased 378.3% compared with untreated checks. This treatment also gave excellent control of annual weeds.

Broomrape on tomato and other hosts in southern California. CARPENTER, T. R. Broomrape, *Orobanche ludoviciana* Nutt. var. *cooperi* (Gray) G. Beck., is an annual root-parasitic angiosperm lacking chlorophyll. It has been reported parasitizing burroweed, *Franseria dumosa* Gray, in desert areas of Southern California, principally in sandy washes. In 1956, it was found parasitizing tomato plants in the Salton Sea area of California. Heavily parasitized tomato plants were stunted and did not produce a full crop of fruit. In 1957, the parasite was found distributed rather widely in the vicinities of Mecca in Riverside County and Niland in Imperial County. Two previously unreported hosts were found in 1957. They were Spanish-needle, *Palafoxia linearis* (Cav.) Lag., and plicate coldenia, *Coldenia plicata* (Torr.) Coville.

Heavy metals and the entrance of glucose into spores of Neurospora sitophila. COCHRANE, VINCENT W., AND DADE L. W. TULL. The inhibition of respiration of spores of *Neurospora sitophila* by uranium and mercury salts constitutes a model system for the study of substrate penetration and surface effects of toxicants. The aerobic endogenous respiration is not inhibited by concentrations of uranium 10 times greater than those that suppress oxygen uptake from glucose. The respiratory quotient in the uranium-glucose system returns to the low level characteristic of endogenous metabolism. Addition of glucose prior to the addition of uranium results in a delay of the inhibition, and inorganic phosphate partially reverses uranium toxicity. Radiochemical studies corroborate these findings and exclude possible substrate effects on endogenous mechanisms; they also show a small uranium-resistant respiration of glucose. Uranyl nitrate at a concentration completely inhibiting glucose respiration has no effect on the oxidation of acetate, ethanol, or glycerol. Mercury, unlike uranium, affects both endogenous and substrate respiration. It is proposed that in *N. sitophila*, as has been shown earlier in studies on yeast, uranium blocks the reactions that occur at or near the cell surface and that are essential to glucose transport.

Relation of plant-growth regulating properties of naphthalene aliphatic acids to their activity in inducing disease resistance. CORDEN, MALCOLM E., AND A. E. DIMOND. A series of naphthalene-substituted aliphatic acids having chain lengths of 2 or 4 carbons varied in growth-regulating properties and in ability to induce resistance to Fusarium wilt of tomato. Compounds were applied as a soil drench to tomatoes prior to inoculation with *Fusarium oxysporum* f. *lycopersici*. At 10^{-5} M, α-naphthaleneacetic acid induced greatest resistance, α-naphthalenebutyric acid (I) and its ethyl ester (II) induced moderate resistance, and α-naphthoylpropionic acid (III) and its ethyl ester (IV) induced slight resistance. β-Substituted naphthalenes corresponding to I, II, III, and IV had no effect on resistance. Growth-regulating activity of all compounds was measured in terms of capacity to inhibit root elongation, promote root initiation, and delay leaf abscission. Only the capacity to inhibit root elongation was related to ability to induce disease resistance; these were directly related. Thus, ability to delay leaf abscission or to promote root initiation is not helpful in reducing disease severity. Assays of the ability of compounds to inhibit root elongation can be used to forecast potency in inducing disease resistance. This growth-regulating function may play a role in resistance of tomatoes to wilt, apart from the relation of root size to inoculum potential.

The significance of Pierce's disease in the decline of bunch grapes in Florida. CRALL, J. M., AND L. H. STOVER. Studies were initiated in 1955 to determine the significance of Pierce's disease in the decline of bunch grapes in Florida. Carrignane vines with shoots exposed to attack by naturally occurring insect vectors developed typical symptoms and were dead within 18 months, regardless of whether they were planted in the field or in pots of fumigated soil. Parallel series of vines, with shoots screened from insects, remained healthy. Series of 10 Carrignane vines were grown in screen cages into which known or suspected vectors were introduced. Percentages of dead vine prunings 9 months after exposure to insect attack were as follows: no insects, 15.8; *Carneocephala flaviceps* Riley, 31.0; *Homalodisca* spp., 61.0; and *Oncometopia undata* F., 73.9. Only 1 of 32 Carrignane vines on which infected scions of Extra were grafted remained healthy after 14 months. There were no symptoms of Pierce's disease in 14 Carrignane vines on which healthy Extra scions were grafted. Results of these studies not only confirmed the presence of the disease but indicated that Pierce's disease is the major factor responsible for decline of bunch grapes in Florida. *O. undata*, and *Homalodisca* spp. (either *H. triquetra* F. or *H. insolita* Wkr., or both) were more effective vectors of the virus than was *C. flaviceps*.

Mechanical and natural transmission of a yellow strain of alfalfa mosaic virus to clones of Atlantic alfalfa. DIACHUN, STEPHEN, AND LAWRENCE HENSON. A strain of alfalfa mosaic virus that causes yellowing, blotching, and stunting was inoculated mechanically to 3 plants of each of 100 clones of Atlantic alfalfa; symptoms were induced in 161 plants in 73 clones. After these inoculated plants were set in the field in April, 1956, in rows alternating with healthy plants of the same clones, symptoms developed in 19 plants (16 clones) during the summer and fall, presumably as a result of field spread. Aphids were abundant on the plants in the fall. In late April, 1957, symptoms developed in 105 additional plants (53 clones). Between April, 1956, and May, 1957, field infection occurred in 124 plants (57 clones); other plants of 14 of these clones had not been inoculated successfully by mechanical methods. Symptoms ranged from inconspicuous small yellow blotches on leaves in some clones to striking yellow blotching, stunting, distortion, and some blotchy leaf necrosis in others. Symptoms induced by this strain of the virus are suppressed at low temperatures and are prominent in the summer. This distinctive strain of alfalfa mosaic virus appears to be useful in studies on natural spread and in the selection of tolerant

or resistant individual plants or clones.

A Penicillium causing wilt in peach and pear seedlings. DIENER, T. O., AND M. L. WEAVER. In greenhouse tests during the spring of 1956, 3 Lovell peach seedlings growing in Hoagland solution wilted and died 4–5 days after being placed in the solution. Seedlings growing in a similar solution containing 10 ppm of As (added as $Na_2HAsO_4 \cdot 7H_2O$) remained healthy. A species of *Penicillium* was isolated from roots of the wilting seedlings. Addition of 10 ppm of As to the medium arrested its growth. The wilt disease could be produced when fungal mats of the Penicillium were added to Hoagland solution in which Lovell peach seedlings were growing. The wilt was favored by hot weather. Pear seedlings (*Pyrus communis, P. calleryana, P. ussuriensis*) growing in Hoagland solution into which Penicillium mats were placed wilted and died. The disease could also be produced in pear, but not in peach, by dipping the roots of seedlings in shredded fungal mats and then planting the seedlings in soil. Check plants and seedlings subjected in like manner to other fungi isolated from roots of wilting seedlings did not wilt. Cucumber, soybean, pea, and Pinto bean plants remained healthy when inoculated with the fungus. Apparently the Penicillium is not parasitic but produces a wilt-inducing toxin.

Reduction and promotion of the development of Fusarium wilt of tomato by gibberellic acid. DIMOND, A. E., AND MALCOLM E. CORDEN. Gibberellic acid (GA) accelerated the growth of tomato plants for 2 weeks after application, but untreated plants eventually became as tall as treated ones. Treatments did not affect root development. One month after tomato plants were inoculated with *Fusarium oxysporum* f. *lycopersici*, Fusarium wilt symptoms in plants sprayed with 5 ppm GA at, or 1–2 weeks prior to, inoculation were less severe than symptoms in unsprayed plants, whereas those in plants sprayed with 10 or 20 ppm were more severe. Thus GA, after its growth effect has disappeared, continues to affect resistance of tomato to Fusarium wilt. In both cases, however, the effect on disease development was greatest when GA was applied on the day of inoculation and least when applied 2 weeks before inoculation. Also, its effect on disease severity contrasts sharply with that of auxin-like growth regulants, which increase wilt resistance of tomatoes as concentration increases. GA had no effect on growth of Fusarium in culture in the range from 1 to 20 ppm.

The inhibition of hatching of nematode eggs under moisture stress. DROPKIN, VICTOR H., AND GEORGE C. MARTIN. Solutions of various salts and of dextrose at a molar concentration equivalent to a potential osmotic pressure of 15 atmospheres prevented the emergence of larvae from eggs of *Meloidogyne* spp. and of *Heterodera rostochiensis*. Development of eggs of *M. arenaria* proceeded normally in 0.3 M NaCl, but the larvae remained motionless until eggs were removed to water. Rates of hatching in water after release from inhibition were inversely related both to concentration of the inhibiting solutions and to the length of exposure. Eggs of *M. javanica* hatched after inhibition by NaCl for as long as 150 days. Root-knot larvae were infective after release from inhibition by 1 M salt solutions. The value for complete inhibition of larval emergence coincides with the moisture stress that brings on permanent wilting of plants. This response of nematode eggs to moisture stress is probably an important factor in the distribution and infectivity of plant parasitic nematodes.

A previously undescribed virus disease of soybean. DUNLEAVY, JOHN. In 1955 and 1956, a virus disease similar to bud blight was observed on soybean. Cross-protection tests and a study of host range showed that it was not tobacco ringspot virus. The virus occurred on *Setaria viridis* and *Melilotus alba* near fields with infected soybean plants. Its host range was the same as that of cucumber mosaic virus, with the exception of certain members of the Leguminosae, when tested on 28 genera of plants in 12 families. Field symptoms in addition to those described for bud blight are necrosis of pod sutures followed by splitting of green pods along sutures. Angular necrotic areas along margins of pods fall out as the pods enlarge. Under greenhouse conditions, symptoms on soybean could not be distinguished from those produced by tobacco ringspot virus. The virus was transmitted through seed of soybean in 3 separate tests.

Physiology of chloroplast breakdown in Saintpaulia. DURBIN, RICHARD D. Chloroplast breakdown in the palisade layer of *Saintpaulia ionantha*, resulting in the physiological ring spot disease, takes place in 2 stages. The first stage appears as darkened areas in the leaf tissue. It is induced within a few seconds when leaf temperature is lowered by contact with cold water or air. Light is not necessary for this stage but may enhance injury by increasing leaf temperature above the ambient. Temperature increase varies with different light sources and is related to the amount of infrared radiation produced by each. In the second stage, chlorophyll in the dark areas is degraded, and yellowish ring patterns and blotches are produced. This stage is light dependent; in the dark, the darkish areas may disappear and the plastids appear to return to normal. In light, photosynthetic rate and total chlorophyll, carotene, and xanthophyll content decline with time. The loss is most rapid during the first 12 hours. Neither high CO_2 or O_2 levels nor the infiltration into the leaves of kinetin, glucose, or oxidizing or reducing agents appear to affect this reaction. The respiration rate of affected tissue does not, however, differ from that of healthy tissue. It is unlikely that the phenomenon involves a photochemical oxidation process such as solarization.

Use of microburet in slide germination tests. EASTBURG, P. H., AND B. L. MCCASKEY. A technique designed for use in spore germination tests makes possible the rapid and accurate application of small volumes of fungicides to glass slides by regulating the plunger movement of a glass syringe with a micrometer. Volumes of solutions thus regulated are applied with a micropipette to the center of a paraffin wax ring prior to the application of spore suspensions.

A mist method for the evaluation of fungicides. EASTBURG, P. H., B. L. MCCASKEY, AND W. D. THOMAS, JR. An apparatus for vertical spraying of fungicides has been devised by which rapid simultaneous tests can be made, with reproducible results, against several fungi. A modified plate-disc technique is used whereby fungus-impregnated paper discs are exposed on agar plates. Spore suspensions atomized onto treated plates also can be employed. The apparatus consists of a variable-speed turntable, 18 in. in diameter, housed in a metal chamber designed to provide a uniform cloud spray at the turntable level. The chamber, with an atomizer at the top, consists of a pipe 6 in. in diameter and 10 in. long, which expands to a cone 20 in. in diameter over a vertical distance of 24 in. The cone empties into a cylinder, 24 in. in diameter and 18 in. high, that is provided with a door. The turntable is at the bottom of this cylinder. Below the turntable the chamber expands into another cylinder, 26.5 in. in diameter and 6 in. high, with a cone that tapers to 2 in. within a vertical distance of 6 in. A reservoir, located at the bottom of this lower cone, is provided for the runoff; it is equipped with a 2-in. drain outlet with and a 10-in. pipe leading to a standard air-evacuation system.

Mechanical transmission of certain stone fruit viruses from Prunus pollen. EHLERS, CLIFFORD G., AND J. DUAIN MOORE. Pollen was collected from Shiro plum and from Early Richmond, Montmorency, and Dyehouse sour cherries known to be infected with various stone fruit viruses. Inoculum was prepared by grinding the pollen in a small quantity of 0.01 M sodium phosphate buffer at pH 8. Mechanical inoculations were made to *Cyamopsis tetragonoloba, Sesbania exaltata, Nicotiana tabacum* (T. I. 787), and

Citrullus vulgaris var. Golden Honey Cream. Symptoms developed on 1 or more plants of those inoculated with a given virus source. Viruses A, B, and E were found to be mechanically transmitted from pollen. It appears probable that virus G also was transmitted. No virus was recovered from inoculum prepared with pollen from a virus-free Montmorency source. The recovery of viruses A, B, E and possibly G from a given source indicated that differential transmission through pollen as a means of obtaining separation may not occur.

The effect of shading and of temperature upon the expression of symptoms in cereals infected with barley yellow-dwarf virus. ENDO, R. M. Shading oat, barley, and wheat plants inoculated with barley yellow-dwarf virus increased the incubation period and decreased the severity of the disease. Shading caused very marked differences in the length of the incubation period and in symptom severity in plants infected with a mild strain and intermediate effects in plants infected with a highly virulent strain. In the temperature studies, plants were incubated at 65°, 75°, 82°, and 88° F. Plants infected with a moderately virulent strain developed symptoms in 19, 14, 14, and 16 days, respectively. Symptoms were severe at 65° and 75° F and progressively less severe at 82° and at 88°. The highly virulent isolate not only caused symptoms sooner (4–7 days depending upon the temperature) than did the mild one, but it killed oat and barley plants at 65°, 75°, and 82°; only moderately severe symptoms developed at 88°. In plants infected with the mild isolate, the incubation period was 3–10 days longer, depending on the temperature, than in plants infected with the virulent isolate. Good symptoms developed only at 65° and 75°, very poor symptoms developed at 82°, and none were present at 88°. Experiments were conducted with the following schedule of day and night temperatures: 70°–65°; 80°–65°; and 88°–65° F. More severe symptoms were produced with all isolates when nights were cool than when they were warm.

Evaluation of resistance in oats to the barley yellow-dwarf virus. ENDO, R. M. During the past 3 years, over 4000 varieties or selections of *Avena sativa* and other oat species have been tested for their reaction to a moderately virulent strain of barley yellow-dwarf virus. Oats were inoculated by infesting plants in the 3-leaf (most susceptible) stage with viruliferous apple-grain aphids. Following a 3-day feeding period, the aphids were killed with a malathion spray. Symptoms developed in 9–18 days. No immune or highly resistant oat was found. At maturity, the plants were grouped into 6 reaction classifications: extremely susceptible, very highly susceptible, highly susceptible, moderately susceptible, slightly resistant, and moderately resistant. Varieties in the extremely susceptible class failed to produce heads, whereas these in the remaining classes usually did so. The extent to which spikelet number and development of grain were reduced determined the 5 remaining reaction classes. All oats developed excellent to good leaf symptoms, except for the moderately resistant Saia and some other lines of *A. strigosa*. Albion (C.I. 72 9), C.I. 4918, and Fulghum (C.I. 1915) possess slight resistance.

Bacterial blast, a newly recognized disease of almonds in California. ENGLISH, HARLEY, JOSEPH M. OGAWA, AND JAMES R. DAVIS. In the early spring of 1954 a serious blasting of almond (*Prunus amygdalus*) blossoms and leaves was observed in a few orchards in the Sacramento Valley. The disease reappeared in 1955 and was epidemic in several varieties in 1956. Symptoms of the disease include a blighting of leaf and blossom buds, a blasting of blossoms and young leaves, a spotting and shot-holing of the foliage, spur killing, and the formation of limited shoot cankers. The only organism consistently isolated from the affected tissues was a green-fluorescent bacterium tentatively identified as *Pseudomonas syringae*. Artificial inoculations of almond blossoms and leaves in the laboratory and of almond leaves in the field have induced typical disease symptoms. Hypodermic injection of the organism into peach and plum branches resulted in the formation of cankers similar to those caused by *P. syringae*, and prick inoculations into red kidney beans resulted in the development of necrotic lesions. The bacterium is similar, if not identical, to the organism causing the bacterial canker and blast disease of stone fruits and pears in California.

The relationship of Rhizoctonia solani to a root rot disease of flax. ERWIN, DONALD C., AND BILL W. KENNEDY. Since 1953 a root disease of flax has been seen in a few fields in the Imperial Valley of southern California during the latter part of the growing season (February to April). Irregular areas of the periderm tissue on affected tap roots become orange in color in contrast to white tissue of healthy roots. Constrictions about 2–3 in. below the ground, or round to irregular sunken lesions, also may appear on the tap roots. Lateral roots from affected plants usually are decomposed. *Rhizoctonia solani* has been isolated from discolored tap and lateral roots. A Rhizoctonia isolate consistently has incited pre-emergence and postemergence death of seedlings, root rot of 4-week-old plants, and an orange-colored rot of the periderm tissue on tap roots of 3- to 4-month-old plants in the greenhouse; Rhizoctonia has been recovered from the diseased roots. In 1 field test in the Imperial Valley, root rot occurred on 5-month-old plants in soil infested with an oat culture of the fungus. Leaves of inoculated plants were more dried and yellow than were those of the check. The evidence indicates that Rhizoctonia may damage flax roots under certain conditions in the field.

Studies on Phytophthora root rot of alfalfa. ERWIN, DONALD C., AND BILL W. KENNEDY. Phytophthora root rot of alfalfa, which occurs in the summer in the San Joaquin Valley and in the winter in the Imperial Valley of California, is incited by *Phytophthora cryptogea*. It is one of the most serious of the stand-depleting diseases of that crop in California. In experiments in the greenhouse, the fungus induced the highest amount of root rot of seedlings at controlled soil temperatures of 22° and 27° C; very little root rot occurred at 17° and none at 32°. *Cicer arietinum* (garbanzo bean) and *Sesbania* sp. (sesbania) also are susceptible to the fungus. Zoospores appeared to be superior to mycelium or any other type of inoculum for experiments designed to test the resistance of alfalfa varieties. Zoospore formation was induced by incubating Phytophthora-colonized alfalfa stems in aerated ½-strength Hoagland's solution for 15–30 hours. In several greenhouse tests, the variety Lahontan has shown some tolerance to the disease.

X-ray studies on the golden nematode, Heterodera rostochiensis. FASSULIOTIS, GEORGE. A preliminary report by Fassuliotis and Sparrow (1955) indicated that larvae in cysts of *Heterodera rostochiensis* Wr. exposed to X rays at dosages of 20 kr. and above and subsequently inoculated on potato or tomato plants produced apparently normal females and cysts but no viable eggs. In a second series of experiments, cysts were irradiated with dosages of 5, 10, 20, 40, 80, 160, and 360 kr. Emergence of larvae from irradiated cysts was comparable to that from nonirradiated controls at the 5 lower dosages. Hatching from cysts receiving 160 kr. was delayed for 7 days, then resumed at a rapid rate. The total number of larvae emerging compared favorably with that from the other treated cysts and controls. An 85% reduction in larval emergence was obtained from cysts receiving 360 kr. Infectivity of irradiated larvae was inversely proportional to the dosage. No mature females were recovered from roots inoculated with larvae receiving 360 kr. Mature females developed from larvae irradiated at all other dosages, but numbers were reduced for larvae receiving 80 kr. and more. Egg production was apparently normal except that eggs from larvae receiving 20 kr. and above eventually died after the cysts had formed. Histological sections of infected roots revealed normal giant-cell production by the irradiated larvae.

Re-evaluation of the etiology of citrus greasy spot. FISHER, FRAN. E. In the course of its 50-year presence in Florida,

greasy spot has been attributed to a number of causes, including an undescribed organism, fungi, rust mites (*Phyllocoptruta oleivora* (Ashm.)), and physiopathic agencies. Within the past decade, greasy spot has come to assume considerable importance, and much sulfur has been applied to control attendant leaf drop on the supposition that the disease is caused by rust mites. This report confirms the findings of Tanaka and Yamada in Japan and establishes the fact that in Florida greasy spot is caused by a fungus. The etiologic agent in Florida is the *Cercospora* stage of a species of *Mycosphaerella*.

A method for detailed genetic analysis of pathogenic loci in rust fungi, and genetic analysis of pathogenicity in Puccinia sorghi. FLANGAS, A. L., AND J. G. DICKSON. The demonstration of suppressed pathogenic factors in the dicaryon of cultures of *P. sorghi* when inbred, and of the nonallelic inheritance of such factors, pointed to the need of inbreeding and isolation of these genes before a race can be established on a genetic basis. The genic status of a locus for pathogenicity in the fungus is detected by inbreeding a culture and comparing the aecial, clonal, inbred recombinants with it on a complement of differential hosts whose single genes conveying resistance are allelic. This procedure narrows the scope of the interaction and reveals hidden pathogenic potentials of a culture towards a discrete, single gene locus for resistance in the host. Four differentially pathogenic cultures were inbred through *Oxalis corniculata*, and 96 subcultures were studied for pathogenicity on 6 corn lines representing 2 gene groups with factors presumably allelic within each group. The 96 sublines segregated into 8 pathogenic types. On the basis of the pathogenic types recovered, gene pools appeared to condition specific pathogenicity in the dicaryon of *P. sorghi* for specific alleles in the host as well as a specific gene-for-gene interaction.

Fruit deformity of strawberry induced by a stigma-parasitizing fungus. FORD, DONALD H., AND STEPHEN WILHELM. An apparently undescribed fungus parasite of stigmas of strawberry flowers is widespread in central and southern coastal California. Experiments carried on in field and greenhouse have demonstrated that this fungus may cause fruit to become misshapen, apparently by preventing fertilization. Growth of the fungus is restricted to stigmas, where limited penetration and intercellular invasion occurs. In moist weather, abundant conidial sporulation occurs on the stigmatic surfaces. Morphological characteristics place the fungus in the Imperfect Family Moniliaceae. In nature, the fungus has been found growing on stigmas of *Fragaria chiloensis* and of *Rubus ursinus* along the central California coast, and it is postulated that spread has occurred from these native hosts to the cultivated strawberry. The fungus has been grown experimentally on stigmas of many additional rosaceous plants but has failed to grow on flowers of 10 other families.

An evaluation of the use of excised leaf grafts in strawberry virus studies. FULTON, J. P. A positive leaf graft did not assure transmission of the viruses present in the donor plant. Transmission varied with each virus; more reliable results were obtained with mild mottle virus than with virus C(epinasty virus) or with latent virus A. Transmission was affected neither by age of the leaf of the donor nor by storage of donor plants at various temperatures between 21° and 36°C for 24 hours prior to grafting. Efficiency was reduced by exposure of donor plants to prolonged periods of light. In an attempt to develop a rapid aid in identifying strawberry viruses, excised leaves were treated in hot water at various temperatures between 28° and 46°C for 10 and 30 minutes prior to grafting. Although epinasty did not develop in many of the plants grafted to leaves that had been treated in hot water above 40°, the results indicate this was related to virus concentration rather than to elimination of virus C by inactivation. Use was made of this technique in separation of components of a virus complex. Mild mottle virus was isolated by the hot-water treatment from a complex also containing virus C. Virus C was isolated from the same complex by holding the grafted plant 14 days at 33°C.

A rapid method for partial purification of some unstable plant viruses. FULTON, ROBERT W. Most mechanically transmitted stone fruit viruses are very unstable in extracts, losing most or all infectivity in 3 hours. Clear, infective preparations were obtained by grinding infected tissue with about twice its volume of calcium phosphate paste in 0.03 M phosphate buffer and centrifuging for 1 minute or less. Infectivity of the supernatant was equal to or greater than that of untreated extracts and remained infectious much longer than did untreated extracts. The method was effective with Prunus viruses A, B, and H and with rose mosaic and apple mosaic viruses. Tobacco ringspot, cucumber mosaic, tomato spotted wilt, and peach yellow bud mosaic viruses apparently were adsorbed and sedimented with the calcium phosphate. Calcium phosphate freshly precipitated from 0.1 M solutions of Na_2HPO_4 and $CaCl_2$ and containing about 90% water gave best results. Extracts in buffers of pH 6.5 to 8.7 yielded infectious supernatants; pH 7.0 to 8.0 was the optimum range. Ionic strength of the buffer was a critical factor. Viruses in distilled-water preparations were adsorbed but could be eluted by resuspension of the calcium phosphate in 0.03 M buffer. Buffer of 0.1 M or higher yielded slightly green supernatants.

The use of chemical indicators in the study of distribution of row-treatment fungicides. GARBER, R. H., AND L. D. LEACH. To study the distribution patterns obtained when materials are sprayed or dusted in the seed furrow, trials were conducted with radioactive rubidium 86, with an oil-soluble safranin dye, and with a fluorescent dye. Radioautographs made from soil profiles taken from the rubidium-sprayed rows, indicated qualitatively where the material was located. When 1 spray nozzle was used in front of a seed-press wheel, the material was concentrated at the bottom of the seed furrow and the soil near the surface was left untreated. One spray nozzle behind a seed-press wheel concentrated the material near the soil surface, leaving the bottom of the furrow untreated. Two nozzles, 1 in front and 1 behind the press wheel, provided more uniform distribution but introduced a mechanical problem because of the wetting of the soil ahead of the press wheel. Because of the difficulties associated with dusting with radioactive materials, oil-soluble safranin was used to study both spray and dust applications. The treated profiles were divided into ½-in.-square sections. The dye was removed from the sections with xylol and measured colorimetrically. Profiles treated with a flourescent dye were observed under ultraviolet light. Preliminary results indicate that this material has possibilities for a rapid evaluation of the distribution pattern.

Developmental and genetic bases of resistance of wheat to loose smut. GASKIN, TIMOTHY A., AND JOHN F. SCHAFER. Resistance of wheat to the loose smut fungus, *Ustilago tritici* (Pers.) Rostr., may function at several different stages of plant development. That maternal tissue of Tremezino (P.I. 191704) inhibits penetration was indicated by differential infection of reciprocal F_1 plants of a cross with the susceptible variety Knox. Exclusion of the pathogen by the embryo in Hope-Hussar (C.I. 11682) was demonstrated by microscopic examination of inoculated seed. Reduced ability of the fungus to invade the growing point following seed germination was observed in Riette (P.I. 191749). Examination of seedlings at 2 stages of development showed that the fungus was unable to maintain itself in the growing point of Kawvale (C.I. 8180) following earlier invasion. Comparison of preliminary genetic studies with developmental studies indicates that there is a relationship between the number of stages of plant development at which resistance is expressed and the number of genes that condition the reaction to the loose smut fungus.

Cultural variants in Ustilago striiformis. GOVINDU, H. C., AND GEORGE W. FISCHER. Single-teliospore isolates from over 40 collections of *U. striiformis* from 34 species in 18 genera of grasses were cultured at a constant temperature of 20°C on a standardized agar medium containing potato extract, sucrose, and malt extract. There was considerable variation among the isolates in cultural characters: topography, color, consistency, and rate of growth. In consistency, 2 types were noted. The sporidial type is smooth, effuse, thin, and whitish, with little or no mycelium. The mycelial type is made up of finely or coarsely tufted hyphae, forming mostly thick and more or less convex colonies. The mycelial colonies show a wide range of color variation among the isolates, as well as sectors of different colors in the same colony. Cultures of several isolates from *Poa pratensis*, *P. alpina*, *Dactylis glomerata*, and *Koeleria cristata* soon develop an abundance of normal-appearing teliospores. Many of the isolates have a distinct odor, in one instance similar to that of a fermenting yeast. Most of the cultures exhibit a counterclockwise or radial pattern of growth, but in a few this is clockwise.

The morphology and chemistry of the teliospore wall of the dwarf bunt organism. GRAHAM, SHIRL O. Mature teliospores of the dwarf bunt organism *Tilletia contraversa* Kühn were treated with varous dyes, enzymes, and reagents. The wall is composed of several morphologically distinct and separable layers. The outer or sheath layer is soluble in 5% alcoholic potash when refluxed over boiling water for 1 hour. It is digested by pectinase. In chemical composition it is a pectin-lipoid complex, possibly containing suberin. The reticulum is soluble in potassium hypochlorite and can be expanded and separated mechanically as a net from the endospore by treatment with 1% sodium hypochlorite followed by 3% potassium hydroxide. Enzymatic digestion and chemical tests indicate that it is a bipectin-protein-hemicellulose-chitin complex. A chitinous compound cements the net to the endospore. The endospore is composed of 2 layers that are mechanically separable after mild oxidation with sodium hypochlorite followed by 3% potassium hydroxide in Shear's mounting fluid. Endospore layers were differentiated with safranin O dye after mild oxidation by the Mäule reaction or with buffered recorcin blue after refluxing for 1 hour in 5% alcoholic potash and digestion in 1% potassium hydroxide. Chemically, the inner endospore layer is a hemicellulose-chitin-pectin complex. The outer endospore layer is chitin.

Inhibition of local-lesion and systemic plant virus infections with a new antiviral agent, cytovirin. GRAY, REED A. A new antiviral agent, cytovirin, produced by a microorganism, was isolated solely because of its activity against plant virus diseases. It was produced and isolated in crystalline form as a result of a group research effort by microbiologists and chemists in the Merck laboratories. Crystalline cytovirin completely inhibited local-lesion formation by southern bean mosaic virus on Pinto bean plants and by tobacco mosaic virus on plants of *Nicotiana rustica* when a spray containing 0.5 ppm of the antibiotic was applied 1 hour after inoculation. When 9 Bonny Best tomato plants were inoculated with spotted wilt virus and then sprayed twice, 1 and 5 days after inoculation, with a solution containing 5 ppm of the pure material, 7 of the 9 treated plants were still protected from the disease 38 days later. All 9 of the control plants succumbed to the disease 13 days after inoculation. A spray containing 5 ppm of pure cytovirin, when applied to White Burley tobacco plants 1 day before inoculation with tobacco mosaic virus and again 1 week later, protected 5 of the 10 treated tobacco plants from the disease for 4 weeks. All 10 control tobacco plants showed systemic symptoms of the disease in 1 week. Cytovirin completely prevented systemic infection with southern bean mosaic virus for 38 days.

The vertical distribution of Verticillium albo-atrum in muck soils and its control. GREEN, RALPH J. Attempts to control Verticillium wilt of commercial peppermint (*Mentha piperita* L.) and of Spearmint (*M. cardiaca* L.) by genetic resistance or chemical amendments have been largely unsuccessful. Various cultural practices have been studied, therefore, as a means of limiting this disease in areas where it exists. Studies of the vertical distribution of *V. albo-atrum* in muck soils of this region revealed a pattern completely different from that found in mineral soils. Soil samples were taken in 6-in. increments to a depth of 36 in. and rooted cuttings of susceptible peppermint plants used as indicator plants. The infection incidence from each sample was as follows: 0 to 6 in., 97.2%; 6 to 12 in., 90%; 12 to 18 in., 52%; 18 to 24 in., 0%; 24 to 30 in., 4.5%; 30 to 36 in., 0%. A soil-inversion technique was developed by which heavily contaminated topsoil can be buried to a depth of 14–18 in. A 36-in. moldboard plow with a 16-in. brush plow mounted on the frame ahead of the large plow was used to turn the top 10–12 in. of soil into the previous furrow before the large plow inverted the remaining 18–24 in. of subsoil. In 1956, 57.5% of the plants on a conventionally plowed area had wilt, whereas only 4.0% of those on an area plowed to a depth of 30–32 in. were wilted. Weed control, increased moisture retention, and reduced soil movement by wind are additional benefits derived from this practice.

Some studies on the nature of the cause of big vein of lettuce. GROGAN, R. G., F. W. ZINK, W. B. HEWITT, AND K. A. KIMBLE. There is no proof in the literature that the cause of big vein of lettuce is a soil-borne virus, although some publications indicate that this relationship has been established. The present studies have demonstrated the following characteristics of the causal agent. Roots from infected plants were rendered noninfectious by surface sterilization for 10 minutes with solutions of 5–1000 NaOCl, 1–1000 $HgCl_2$, or 1–1000 Semesan (28.6% 2-chloro-4-(hydroxymercuri) phenol), but not by 1–100 Vancide 51 (30% sodium dimethyl dithiocarbamate and sodium derivative of mercaptobenzothiozole), 1–100 nabam, or 1–100 captan. Plants grown in infested soil for 8 weeks failed to develop symptoms when they were removed from the infested soil, washed in water, immersed in 1–2000 $HgCl_2$ for 10 minutes, and transplanted into steam-sterilized soil. Similarly grown plants that were merely washed in water but not treated with $HgCl_2$ developed big vein symptoms in most cases. The causal agent of big vein settled to the bottom of a 1–1 suspension of infested soil and water that was allowed to stand overnight. The causal agent passed through a filter with a 40-μ pore size but not through one with a 14-μ pore size or smaller. The superficial scrapings from washed big-vein-affected roots were infectious, but the interior portions of the same roots were not.

Effects of postinoculation environment on virus infection in clovers. HAGEDORN, D. J., AND E. W. HANSON. The effects of air temperature and length of day during the 10-day period following inoculation of alsike clover, red clover, crimson clover, and yellow sweetclover with alfalfa mosaic, red clover vein mosaic, and bean yellow mosaic viruses were determined. Plants inoculated with each virus were kept at 15°, 20°, or 25°C and given 4, 8, or 16 hours of light (4800 foot-candles) per day. After the test period, all plants were treated alike. The effects of the treatments varied with the virus and host. Alfalfa mosaic virus infected the largest percentage of plants of alsike and crimson clovers at 25° and 16 hours of light, of sweetclover at 20° and 16 hours of light, and of red clover at 15° and 16 hours of light. Red clover vein-mosaic virus infected the largest percentage of plants of alsike and sweetclover at 20° and 16 hours of light, of red clover at 20–25° and 4 hours of light, and of crimson clover at 15° and 4 hours of light. Preliminary tests with the bean yellow mosaic virus indicate that the largest percentage of infections of sweetclover and alsike clover occur at 20°. In most cases, each of the viruses produced good infection over the entire range of conditions studied.

Effect of seed treatment with fungicides and with combinations of fungicides and insecticides on emergence and control of covered kernel smut of sorghum in 1956. HANSING, E. D. An experiment on emergence was conducted with the sorghum varieties Atlas, Midland, and Westland and the DeKalb hybrids C-44, D-50, and F-62. The treatments were randomized within each of 5 replications, and 200 seeds were planted per row. The average emergence for the non-treated seed was 46%, whereas that of seed treated with Panogen 15 (2.2% cyano(methylmercuri)guanidine) was 53% and that of seed treated with Ceresan M (7.7% *N*-ethylmercuri)-*p*-toluene sulfonanilide), 51%. The emergence of seed treated with Panogen 15 and Drinox (30% aldrin) was 60%. Emergence for seed treated with non-mercurials was as follows: Arasan SF-X (75% thiram), 54%; Panoram 75 (75% thiram), 54%; and Captan 75 (75% captan), 52%. Sixty-one per cent of seed treated with Panoram D-31 (56.2% thiram and 18.8% dieldrin) emerged. In an experiment in which untreated Pink kafir seed was inoculated and planted in 3 replications, an average of 40% of the heads were smutted. Complete control of smut was obtained with the above seed treatments. Control also was obtained with combinations of thiram-dieldrin, captan-dieldrin, and captan-lindane. Higher emergence was obtained with the combinations than with their fungicide counterparts alone.

Fungi associated with oat seed in Kansas. HANSING, E. D., C. T. ROGERSON, AND R. W. BEARDMORE. A total of 156 farmers' samples of seed were selected at random from the 1950 through 1953 crops of the oat varieties Kanota, Fulton, Osage, Neosho, Cherokee, and Nemaha. During the winter and spring of 1954, seed of each sample was surface sterilized and then 100 seeds of each plated on acidified potato-dextrose agar. The isolates obtained in the different classes of fungi were 2 Phycomycetes, 1843 Ascomycetes, 729 Deuteromycetes, and 99 unknowns. The isolates were distributed among 6 orders, 11 families, 30 genera, and 50 identified species. *Chaetomium olivaceum* was isolated most frequently, accounting for 793 isolates, with the majority from the 1950 seed. Isolates of *Alternaria tenuis* were second, with a total of 475, of which the majority was from the 1953 seed. Other fungi isolated 10 or more times in order of prevalence were *Chaetomium globosum* (285), *Sordaria fimicola* (145), *Eurotium chevalieri* (128), *Chaetomium indicum* (128), *Thielavia terricola* (83), *Chaetomium elatum* (69), *Sporotrichum roseolum* (62), *Chaetomium dolichotrichum* (49), *Eurotium amstelodami* (48), *Cephalosporium asperum* (29), *Fusarium roseum* (21), *Xylaria* sp. (17), *curvularia geniculata* (14) *Microascus cirrosus* (13), *Sporormia australis* (13), *Pullularia pullulans* (13), *Helminthosporium sativum* (12), *H. spiciferum* (12).

A new approach to the evaluation of nematocides for the control of the golden nematode. HARRISON, MARTIN B. In the golden nematode research program, the testing of compounds such as D-D (1,3-dichloropropene; 1,2-dichloropropane) and E.D.B. (1,2-dibromoethane) as nematocides has been performed in 1-gal. glazed crocks filled with infested soil. Efficacy was determined by a larval emergence test on cysts removed from the treated soil 2 weeks after fumigation. The introduction of several new nematocides of low phytotoxicity and low vapor pressure has permitted a modification in the test methods. The experimental nematocide PRD (3,4-dichlorotetrahydrothiophene 1,1-dioxide) was tested in 4-in. clay pots in which potato plants were grown. The test compound was added to the soil at the time of planting and at intervals thereafter. A count of the immature females that developed on roots at the periphery of the soil ball was taken as an indication of the efficacy of the compound. The average number of immature females from the 3 replications of each treatment was as follows: PRD (40 lb./acre), 1.6; PRD (80 lb./acre), 1.3; PRD (160 lb./acre), 0.6; D-D (450 lb./acre), 13; and untreated, 70. Data on phytotoxicity may be taken simultaneously with counts of female nematodes.

Relation of field infection to decay in stored Emperor grapes. HARVEY, JOHN M. Incipient field infections are the primary cause of spoilage in stored Emperor grapes. Post-harvest infection is largely prevented by sulfur dioxide fumigation. The measurement of infections present at harvest has been used to forecast decay in storage. Grapes harvested within 2 weeks after exposure to rainfall suffered severe decay in storage; this was consistent with forecast at time of harvest. Those harvested 3 weeks after exposure had a negligible amount of decay in storage, since infected berries were detected and removed at harvest. The forecast predicted variations in potential decay in individual lots as related to harvest date and vineyard source. This information was used as a guide to marketing and to reduction of spoilage losses in storage. *Botrytis cinerea* was the principal cause of decay in grapes harvested after rainfall. Rots caused by *Cladosporium herbarum* and *Alternaria* sp. were less prevalent and were related less to rainfall than was *B. cinerea*. In dry seasons, *C. herbarum* was the predominant cause of decay. Counts of spores present on the surface of grape berries at harvest indicated no relation between storage decay and spore population.

Relationship of stomatal size and number in gladiolus to varietal response to atmospheric fluorides. HENDRIX, J. W., AND H. R. HALL. The relative sensitivity of 42 varieties of gladiolus was determined by making planimeter measurements of the extent of foliar scorch on a series of leaves systematically selected from plants grown within a fluoride pollution area near Spokane, Washington. Stomatal frequency and the size of stomatal wells were determined from corresponding leaves selected from plants grown near the periphery of the pollution zone. In the latter determinations, both microscopic cross sections and celloidin impressions taken at 12 predetermined leaf positions (front and back surfaces) were utilized. These studies revealed 1) wide differences in varietal sensitivity to atmospheric flourides (33–85% of total leaf area scorched); 2) wide differences in average stomatal frequency among varieties (145–257 per sq. mm); and 3) noticeable differences in the average diameter of stomatal wells (6.78–15.74 μ). A plotting of these results reveals a fair, but necessarily constant, correlation between varietal reaction and each of 2 factors: stomatal frequency and stomatal well diameter. In general, low stomatal frequency and/or small well size were associated with resistant varieties whereas the more sensitive varieties tended to possess higher stomatal frequency and/or greater well size.

Resistance to Helminthosporium dictyoides in inbred lines of Festuca arundinacea. HENSON, LAWRENCE, AND ROBERT C. BUCKNER. Three families of tall fescue selected for palatability were clonally increased and transplanted to the field in April, 1956, for seed production. One second- and 2 third-generation families were represented by 14, 15, and 15 inbred lines, respectively. The planting consisted of 12 3-plant replicates of each clone, with the plants spaced 3 ft. apart in a randomized block design. In the spring of 1957, an epidemic of net blotch (caused by *H. dictyoides*) developed over the entire planting, providing an opportunity to observe the resistance of these clones to this disease. The most resistant clones were characterized by a few small lesions per plant, but 95–100% of the leaves of the most susceptible plants were infected. On April 12, 1 plant of each replication of each line was scored 1, 3, 5, 7, or 9, the score increasing with susceptibility. The score of the 14 clones of the second-generation family 61-48 varied from 2.7 to 5.5, with an average of 4.0. Scores for clones of the third-generation family 42-33 varied from 2.3 to 8.7, with an average of 6.4. The other third-generation family, 45-50, was highly resistant: 13 and 2 of the clones scored 1.0 and 1.2, respectively.

Isolation of cork virus from symptomless roots of Nemagold sweetpotato. HILDEBRAND, E. M. That the foliage and

root lesions of sweetpotatoes with internal cork contained virus was first demonstrated in March, 1955, when they indexed positively on Scarlett O'Hara morning glory. Since then, this virus has been found associated with all chlorotic spot foliage and necrotic root lesions on all tested sweetpotato varieties wherever grown. An indexing experiment involving roots of several diseased varieties was started after the 1956 harvest. The first test in October demonstrated the absence of root lesions in the varieties–Allgold, Heartogold, Nemagold, Unit I Porto Rico, and Kandee; however, the symptomless Nemagold roots indexed positively for virus. When grown in pots, sprouts from 2 Nemagold sources and from the other varieties all showed typical foliage symptoms (chlorotic spot, ringspot, feather, etc.) as in the field. After 4 months' growth in the greenhouse, the roots harvested were all symptomless, but again Nemagold roots of all sizes indexed positively for virus. After 6 months' high -temperature storage the field-grown roots were sliced and indexed with the result that all symptomed roots indexed positively regardless of variety. Also, although only 1 root had cork lesions, all Nemagold roots indexed positive.

Growth repression of pear trees planted following plum. HINE, RICHARD B. A repressed growth condition in pear trees in old plum soil appeared where plums on Myrobalan rootstock had been removed and pears planted. The condition has persisted for at least 15 years. In an adjacent block, pears planted following peaches grew normally. Soil samples, collected from the 2 adjacent orchards, were planted to dormant pear seedlings and 3-week-old growing pear seedlings. After 2 weeks, the seedlings were removed from the soil and observations and isolations made. Root development of seedlings in soil from the affected orchard was poor, and there were numerous lesions on feeder roots; in contrast, roots of seedlings in the other soil were well developed. Pythium isolates that proved to be pathogenic to pear roots were consistently recovered from the lesioned areas, whereas similar isolations from the roots in soil from the peach orchard yielded mostly Fusarium that did not appear to be pathogenic. Carbon bisulfide applied at 3300 lb. per acre gave no response, whereas steam treatment at 3 lb. per sq. in. for 2 hours eliminated the root rot condition in greenhouse tests. Methods to determine damping-off potential of the soils by indexing with susceptible vegetables revealed a higher potential in the soil from the plum orchard than in that from the peach orchard. Examination of soil and root samples by nematologists failed to reveal pathogenic species of nematodes that might account for the problem.

Use of novobiocin for isolation of fungi from the soil. HINE, RICHARD B., AND EDWARD E. BUTLER. When soil-dilution techniques were used to compare the fungal populations of different soil samples, it was found that potato-dextrose agar supplemented with the antibiotic novobiocin at a concentration of 100 ppm eliminated soil bacteria, reduced radial growth of fungi, and compared favorably with peptone-dextrose agar supplemented with streptomycin and rose bengal as to number and kinds of fungi isolated. The medium was prepared with the aqueous extract of 200 g of potatoes and 10 g of dextrose per liter; it should be used at pH 5.6 to 6.1. More fungi could be identified on the original plates containing novobiocin than on those containing rose bengal and streptomycin. This material has been employed successfully to exclude bacteria in assays of more than 20 different soils and other biological materials. More than 75 species of fungi belonging to the Fungi Imperfecti, Ascomycetes, Basidiomycetes, and Phycomycetes produced colonies on potato-dextrose agar containing 100 ppm of the antibiotic. The only fungi completely inhibited at this concentration were a limited number of species of *Pythium* and *Phytophthora*.

The life cycle and role of intersex in Ditylenchus triformis. HIRSCHMANN, HEDWIG. In addition to normal females and males, *Ditylenchus triformis* produces intersexes when reared in fungus cultures such as species of *Fusarium*, *Helminthosporium*, and *Rhizopus* on potato-dextrose agar. The life cycle, from the egg to the adult nematode, is completed in 16–18 days at 22–24°C. Four molts occur, the first taking place within the egg. The range of body size does not overlap in the subsequent 3 molting stages. The life span of the adult nematode averages 60–70 days but may exceed 100 days. Females lay from 50 to 100 eggs. The intersexes function as females and lay eggs only after copulation with males. Crosses of males × females and males × intersexes revealed that 2 different lines exist within the species. One line possesses only females and males, the other possesses females, males, and intersexes. Crosses of intersexes × males yielded large numbers of intersexes and males but very few females. When these females were mated with males of the same line, again many intersexes and males were obtained but few females. When the males were crossed with females from the bisexual line, however, a population of only males and females resulted.

A statistical study of nematode populations in soil fumigation experiments. HOLLIS, J. P. Populations of *Pratylenchus* spp., *P. zeae*, *Trichodorus* spp., *Tylenchorhynchus acutus* + *T. latus*, and *T. martini* in replicated soil fumigation tests were periodically extracted and counted by standard methods. Low numbers, including 0, were transformed to $\sqrt{X + 0.5}$ and to log $(X + 1)$; high numbers were transformed to $\sqrt{X}$ and to log X. The transformed data were studied in 290 analyses of variance with I.B.M. facilities. Square root and log transforms were generally equivalent with respect to the percentage of tests yielding 1) significant intraseasonal (4–6 week) fluctuations in populations and 2) significant population reductions by fumigants during the crop season. Number of significant analyses at the 1% level was more than double that at the 5 per cent level only. The F value was not related to the average sample or to the sample of the highest nematode number in an experiment. Significant intraseasonal fluctuations and crop-season reductions in populations of nematodes occurred in 41.5 and 55.4% of analyses, respectively. These methods provide data on population dynamics and ecology of common plant parasitic nematodes.

Action of fumigants on nematodes as related to nematocide specifications. HOLLIS, J. P., M. J. FIELDING, AND E. J. WEHUNT. Actions of 4 fumigants on populations of 5 genera of plant parasitic nematodes were analysed in 22 field tests. Fumigants, with vapor pressure in mm of Hg at 20°–25°C, were Dowfume MC-2 (bromomethane; trichloronitromethane), 1628; D-D (1,3-dichloropropene; 1,2-dichloropropane), 29; Dowfume W-85 (1,2-dibromoethane), 12; and Nemagon (1,2-dibromo-3-chloropropane), 0.8. Their effectiveness varied directly with vapor pressure in initial nematode kills (after 2 weeks) and inversely with vapor pressure in reducing populations (all season). In respect to ability of populations to recover after treatment (all fumigants), the genera ranked as follows: weak—*Tylenchorhynchus*; moderately weak—*Pratylenchus*; and strong—*Trichodorus*, *Tylenchus*, and *Psilenchus*. Ability of species of *Tylenchorhynchus* and *Pratylenchus* to recover from treatment varied directly with vapor pressure; that of species of *Trichodorus*, *Tylenchus*, and *Psilenchus* appeared to be independent of vapor pressure. Present-day fumigants are specialized, quick-killing agents lacking persistence; their differential effects depend upon their relative vapor pressures. An ideal nematocide (one of maximal effectiveness and minimal cost) would possess low solubility in water and high persistence in soil and would decompose slowly to yield an active product of low vapor pressure.

Haploid lethal factors associated with two unique sorus types in the oat smut fungi. HOLTON, C. S., AND SHERL M. DIETZ. Lethal factors operative against haploid development in culture were found in association with 2 distinct sorus

types in the oat smut fungi, 1 in the buff smut fungus and the other in *Ustilago avenae*. The former sorus is a covered indurate type that usually is confined to the apical floret and only rarely involves the other florets, which otherwise are rendered sterile. In contrast, the sorus of *U. avenae* is an exposed, indurate type that envelops not only the entire inflorescence but the flag leaf as well. In the great majority of germinating spores from the unique sorus of the buff smut fungus, half of the sporidia exhibit a lethal characteristic that prevents growth in culture. Occasionally this lethal effect is exhibited by 3 or all 4 sporidia from a promycelium and rarely by none or only 1. In spores from the flag leaf sorus produced by *U. avenae*, the lethal effect usually is exhibited by all 4 sporidia from a promycelium, occasionally by only 3, and rarely by only 2. In the buff smut fungus, the lethal factor segregates independently of the 2 sex compatibility factors. In the biotype of *U avenae* that produces the covered indurate sorus, a specific association between the lethal and sex compatibility factors is indicated.

Antibiotics eliminate systemic downy mildew from hops. Horner, C. E., and C. R. Maier. Streptomycin sulfate (as Agristrep) and griseofulvin at high concentrations eliminated downy mildew from systemically infected hop shoots. In 1 test, streptomycin spray at concentrations of 1000, 3000, and 5000 ppm transformed 16, 71 and 100%, respectively, of treated shoots into healthy vines. In another test with a greater volume of streptomycin spray at 0, 250, 500, 750, 1000, 1500, and 2000 ppm, the percentage of hills remaining infected was 91, 69, 59, 34, 11, 9, and 3, respectively. Comparison of 1000-ppm streptomycin spray and dust on 800 hills each resulted in 86 and 0% control, respectively. Application of streptomycin sprays during rapid vegetative growth appeared to result in better control than application at other times. Griseofulvin spray applied at 500 or 1000 ppm to basal shoots eliminated further production of systemically infected shoots for the remainder of the growing season. The following spring, the block of 300 hills previously treated with griseofulvin had 20 infected shoots whereas 300 untreated hills had 141.

Newly determined races of the barley scald fungus in California. Houston, Byron, R., and L. J. Ashworth, Jr. The barley variety Atlas 46 (C.I. 7323) was released to California growers in 1947 as a variety resistant to scald (caused by *Rhyncosporium secalis*) and to powdery mildew (caused by *Erysiphe graminis* f.sp. *hordei*). The variety carried genes for agronomic characters similar to those of Atlas (C.I. 4118) and genes for scald resistance from the variety Turk (C.I. 5611-2). In 1953, the scald disease was found on Atlas 46 in several field locations. By 1956, the variety was extremely susceptible in all areas of the state. Many isolates of the scald fungus were obtained from several barley varieties from widely separated areas in California. The pathogenicity of 28 isolates was determined on the varieties Atlas, Atlas 46, Turk, Trebi, La Mesita, Osiris, and Telli by inoculation of seedlings in the greenhouse. At least 5 physiologic races were found in this group of isolates. With 1 exception, all isolates pathogenic on Atlas 46 were also pathogenic on Turk. One isolate was pathogenic on all 7 varieties. The most common isolate was pathogenic on Atlas only. There was striking variability in cultural characters and sporulation.

Halogen-aceto compounds as fungicides. Howard, F. L., and Barbara C. Cormier. Twenty-five nonmetallic fungicides of potential value in plant pathology were disclosed by a study of 35 compounds of the generic group embodying the iodoaceto, chloroaceto or bromoaceto moiety, e.g. 1,4-bis-bromoacetoxy-2-butene, butane-1,4-diol bromoacetate, and 2-bromoacetoxyethyl chloride. Usefulness as foliar or soil fungicides was determined by laboratory, greenhouse, and field tests. Seven materials inhibited spore germination ($I.D._{50}$) of *Curvularia* sp. at 1 ppm by the A.P.S. standard glass-slide technique. Solutions of 15 compounds at concentrations of 100 ppm or less killed mycelium of *Pythium aphanidermatum* or of *Rhizoctonia solani* after a 1-minute exposure. Twelve compounds at 10 ppm or less inhibited growth of the same pathogens 100% as determined by the Defense Agencies agar-plate technique. No injury resulted when butane-1,4-diol bromoacetate was applied in solution up to 2000 ppm to tomato foliage; however, all other compounds caused injury at concentrations of 500 ppm and upwards. The influence of molecular structure on fungitoxicity was evident.

Factors relating to the water-soak method of plant disease control. Ivanoff, S. S. In studies on the effect of the water-soak method on the control of fungi on unshelled peanuts and of seedling blight of oats (caused by *Helminthosporium sativum*), unshelled peanuts and oat seeds were soaked for 20 hours at room temperature in standing water, with and without addition of 2.5% NaCl, and in sea water. The most important factor in disease control by the water-soak treatment seemed to be the removal from the substratum (seed coats, glumes, or shells), through the process of diffusion, of nutrients necessary for germination and growth of the pathogens. Restoration of nutrients to the previously water-soaked substratum resulted in resumed fungus action. Removal of inoculum is assumed to play a part. Deficiency of oxygen for host and parasite was of some importance in reducing fungus growth and in totally inhibiting germination of seeds that were heavily infected or of low germinability. Antibiotic action by microorganisms was of little or no value in suppressing pathogens in the cases studied. The healthiest seedlings were those produced from seeds soaked in salt water or in sea water. Salts at osmotic concentrations inhibited fungus growth and eliminated "weak" seeds, causing death through cell plasmolysis.

Powdery mildew intumescences on watermelon fruits. Ivanoff, S. S. Overgrowths on the rind of watermelon fruits, in the shape of warts, pimples, scabs, and similar excrescences, werr found to be caused in the greenhouse by a species of *Erysiphaceae*, probably *E. cichoracearum*. Powdery mildew appeared on young fruits soon after they were formed. The fungus colonies resembled on the whole common powdery mildew spots on cantaloupe vines growing adjacent to the watermelons. From 7 to 10 days following the appearance of the mildew spots on the fruits, the areas covered by the fungus became raised. The borders of the raised areas followed almost exactly the outline of the mildew spots. There were no intumescences formed where there was no preceding growth of the fungus. The fungus growth began to disappear even before the intumescences were fully formed. The number of the individual overgrowths on a single fruit varied from a few to over a hundred. The size and form also varied from small (2 mm or less) symetrically shaped "pimples" to flat elevations resembling russetting. Dipping young fruits in 1:2000 Karathane WP (25.5% 2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and 2.5% isomers) prevented infection and hyperplasia.

Further studies on microbiological reduction of nematode population in water-saturated soils. Johnston, Titus. *Tylenchorhynchus martini* and associated nematodes were added to a rice-field soil and incubated at moisture levels of 11, 25, 50, 75, and 100% of field capacity and at saturation. Survival, determined at 3-day intervals, was highest between 40 and 60% and lowest at 11% and at saturation. Population reduction, averaging 75%, usually occurred within 6 to 9 days. Sterile and nonsterile soil samples were adjusted to moisture levels of 50% of field capacity and saturation. In certain sterile and nonsterile saturated series, the oxygen was replaced by nitrogen. Reduction of nematode number was greatest in the nonsterile saturated treatments without oxygen; there was no reduction at 50% moisture. Thirty aerobic and 6 anaerobic bacteria were tested for effects on the nematodes. Nematodes were submerged in the bacterial cultures, filtrates, and media. A species of *Clostri-*

dium produced a toxic principle that killed the nematodes in from 15 seconds to 2 minutes. Activity of the substance was partially reduced by filtration, by agitation for 15 minutes, and by heating at 100°C for 15 minutes. Anaerobic bacteria may be a factor in the reduction of nematode populations in water-saturated soils.

Susceptibility of rice to systemic infection by three common cereal viruses. KAHN, ROBERT P., AND OTTIE J. DICKERSON. Eighteen varieties or plant introductions of rice were inoculated mechanically when 2–3 weeks of age with either barley stripe, brome mosaic, or wheat streak mosaic virus. Inoculated plants were incubated at greenhouse temperatures between 70° and 75°F for approximately 3 weeks and then observed for systemic infection. None of the the varieties was susceptible to infection by 3 isolates of the wheat streak virus. Three varieties (P.I. 201902, 201903, and 231126) were susceptible to 2 isloates of brome mosaic virus. These same 3 varieties plus 4 others (P.I. 184675, 184676, 184676A, and 201907) were susceptible to 1 isolate of barley stripe virus that apparently was free of contaminating viruses. None of the varieties, however, was susceptible to barley stripe virus obtained from plants grown from infected seed. The viruses could be transmitted readily back to cereal plants from rice plants showing systemic infection; none was recovered from the symptomless rice plants. These reactions suggest that certain varieties of rice might be useful as differential hosts for the indentification of mechanically transmitted cereal viruses.

Mechanical transmission of yellow bud mosaic virus. KARLE, HARRY P. Peach yellow bud mosaic virus has been mechanically transmitted to some members of the following families: Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Geranaceae, Leguminosae, Malvaceae, and Solanaceae. Of the many different plants inoculated, Bountiful bean and Virginia Blackeye cowpea are probably the best indicator hosts because they show both necrotic local lesions and systemic symptoms—vein clearing and necrotic lesions. Turkish tobacco is a good local lesion host. No symptoms appeared in, nor could the virus be recovered from, inoculated plants in the Convolvulaceae, Cruciferae, and Umbelliferae. All inoculations were made with diluted juice extracted from Bountiful bean leaves with systemic shock symptoms. All recovery inoculations were made on Bountiful bean or Virginia Blackeye cowpea. Even though naturally occurring orchard weeds such as chickweed, filaree, and wild lettuce became infected when mechanically inoculated under greenhouse conditions, the virus was not recovered from weeds exhibiting symptoms similar to those of the known yellow-bud-affected trees under which they were growing.

The production of teliospores of Tilletia caries in culture. KENDRICK, EDGAR L. Although *Tilletia caries* (DC.) Tul. is readily cultured in the haploid stage on artificial media, growth in a living host plant has been essential for the production of teliospores. In recent studies of 4 monosporidial isolates from germinating inbred-line spores of Race T-16, teliospore production was observed in culture. Two of these spore-producing isolates exhibited the normal secondary sporidial growth of haploid lines; the growth covered the face of the agar slant. The masses of teliospores appeared at random on the surface of this growth. In contrast, the only visible growth made by the other 2 isolates was that of the black spore mass. The spores produced in culture germinated like normal teliospores, producing promycelia with whorls of primary sporidia at the tip. Since the dicaryotic stage is required for teliospore production, these teliospore-producing cultures must have been more than merely haploid lines. This was further indicated by the fact that secondary sporidia from 2 of them would not mate in culture with known haploid plus or minus spordia. Thus, the behavior of these 4 monosporidial lines suggests that they are either dicaryotic or true diploid lines capable of saprophytic propagation.

Studies with virus incitants of pea streak. KIM, WOON S., AND D. J. HAGEDORN. A collection was made of 47 isolates of viruses inciting pea streak in various parts of the United States, and the isolates were mechanically inoculated into peas and other hosts. On the basis of symptomatology and differential host reaction, isolates were placed in 5 groups; an isolate typical of each group was chosen for detailed study. Isolate I-5 (Ida.) was similar to the Wisconsin pea streak virus with respect to symptoms incited and to certain physical properties but differed in host range, temperature effects, and aging *in vitro*. Isolate I-7 (Ida.) was similar to alfalfa mosaic virus but differed somewhat in that it produced a yellow-margined red mottle on soybean, incited local lesions on tobacco, and had a higher dilution end point and a higher thermal inactivation point. Isolate M-S (Minn.) had a dilution end point greater than $1{:}10^6$ and produced characteristic streak symptoms on peas. An unidentified isolate from New York rarely incited streaking on greenhouse-grown peas but caused tip blight of peas and of Crotalaria. The V-III (Wis.) isolate incited severe streaking on peas; although systemic symptoms on zinnia, beans, and tobacco plants somewhat resembled those produced by strain 14, of cucumber mosaic virus, it differed in symptoms on cowpea, host range, physical properties, and temperature effects.

A new staining technique for in situ observation of Pratylenchus penetrans and other endoparasitic nematodes. KIRKPATRICK, J. D., AND W. F. MAI. A method has been developed by which various stages of endoparasitic nematodes can be clearly differentiated from root and other host tissues. Clearing by means of a bleach makes possible the use of a wide variety of stains. Advantage is taken of the buffering capacity of proteinaceous material to distinguish between plant and nematode tissues. Material containing endoparasitic nematodes is placed in 0.5–1.0% $NaOCl_2$ for 4–8 hours, rinsed in water, and immersed in 50% ethanol for several minutes. When drained, it is placed in either 1) bromphenol blue or bromthymol blue, 1% solution in 50% ethanol, for 4 hours or more to develop maximum contrast or 2) in a 0.05–0.10% solution of the same dye in 50% ethanol for 12–16 hours for greater detail. When drained and rinsed, the material can be observed in 0.2% acetic acid in 50% ethanol, or it can be transferred to water for several minutes and mounted in glycerol, or it can be transferred rapidly through a series consisting of 50% ethanol, 70% ethanol, and 70% isobutanol (1–2 minutes in each) and mounted in diaphane. Use of heated cover slips reduces bubble formation. The nematodes and eggs are green to deep blue in colorless tissue. Placing the material in 1–50,000 safranine prior to mounting increases cell wall detail. After 10–14 days, the colors fade but can be reconstituted by immersion in the dye.

The toxic effect of quackgrass on growth of alfalfa. KOMMEDAHL, T., A. J. LINCK, AND J. V. BERNARDINI. In field tests, both stands and dry weights per plant of alfalfa were half as great when alfalfa was sown in soil previously occupied by quackgrass, *Agropyron repens*, as when sown in quackgrass-free soil. In the greenhouse, the addition to soil of dried rhizomes from quackgrass (ground to a 40-mesh size) resulted in 1) severe stunting and chlorosis of alfalfa seedlings, 2) reduction in stand of alfalfa seedlings, and 3) a delay in germination of alfalfa seeds. A second planting of alfalfa in soil mixed with ground rhizomes also resulted in stunted alfalfa plants. Ground rhizomes were mixed with water and filtered. The green weight of alfalfa grown on soil to which the residue and filtrate were added separately was only 20 and 75%, respectively, that of plants grown on untreated soil. Autoclaving of either soil or rhizomes did not change the results appreciably, and the alfalfa varieties Narragansett and Ranger were affected equally.

Effect of virus infection on flowering and seed production of Ladino white clover. KREITLOW, K. W., AND O. J. HUNT.

In greenhouse tests, 31.7% fewer flower heads were produced on 320 plants of a virus-infected clone of Ladino white clover than on an equal number of healthy plants of the same clone. When healthy and virus-infected plants of 2 clonal lines were compared in field plots, the virus-infected plants produced 43.9 and 20.3% fewer flower heads per sq. ft. than did healthy plants of the respective clones. Seed yield from virus-infected plants of the 2 clones was 53.7 and 29.2% less, respectively, than that of their healthy counterparts. There was no difference in germinability of seeds harvested from virus-infested and from healthy plants, nor was there any difference in percentage of abnormal seeds. Virus was not transmitted through seeds harvested from infected plants.

Inhibition of tobacco mosaic virus multiplication and effects on host tobacco tissue cultures by substituted purines. KURTZMAN, RALPH H., JR., ALBERT C. HILDEBRANDT, ROBERT H. BURRIS, AND A. J. RIKER. Several 6-substituted purines and 2-oxypurines were tested for activity against tobacco mosaic virus multiplication in infected tissue cultures of *Nicotiana tabacum* L. Virus activity in the cultures, after incubation for 1, 3, and 6 weeks on media containing the analogue, was measured with a local-lesion assay on a hybrid of *N. tabacum* × *N. glutinosa*. Host tissue growth was measured as wet weight after 6 weeks as an index of phytotoxicity. Virus multiplication was inhibited by 6-methylpurine at 0.01 mg/l; inhibition was progressively greater over the range of 0.05, 0.1, 0.25, 0.5, 1, 10, and 100 mg/l and approached 50% at the 100-mg level. Phytotoxicity occurred at 0.25 mg/l, and no growth occurred above 0.5 mg/l (25-mg tissue seed pieces). The 6-chloropurine was neither antiviral nor phytotoxic at levels of 10 mg/l or less, but above this level it was phytotoxic and had antiviral activity. At 15, 22.5, and 33.7 mg/l, both tissue growth and virus infectivity decreased progressively. Fifty and 100 mg/l allowed no growth of the host tissue, but virus infectivity was reduced to about 50% of that of the control. Hypoxanthine and 6-mercaptopurine (0, 0.01, 0.1, 1, 10, and 100 mg/l) and 2-oxypurine (0, 0.01, 0.1, 1, and 10 mg/l) showed no antiviral activity or phytotoxicity.

Three new naturally occurring strains of wheat streak mosaic virus in Kansas and the Great Plains. LAL, S. B., W. H. SILL, JR., MARIA S. DEL ROSARIO, AND J. M. KAINSKI. Of 414 samples of wheat streak mosaic virus collected in wheat and other grasses in the Great Plains during 6 years, 360 were the yellow strain (*Marmor virgatum* var. *typicum* McK.); 52 were of 4 other symptom types, including that of the green strain (*M. virgatum* var. *viride* McK.). Nine were mixtures of viruses of 2 or more symptom types. Viruses of each of the 5 types were purified by selection for specific symptoms and by repeated transfers to Pawnee wheat. Each had physical properties similar to those of the yellow strain, and each was transmitted by the mite vector, *Aceria tulipae* (Keifer). The green strain protected Pawnee wheat from infection by the other 4. The viruses of the 3 new symptom types are designated as the white mottle, leaf rolling, and leaf striping strains. Search for diagnostic hosts among 50 wheat varieties, 20 Agropyron × wheat hybrids, and all other known hosts showed that Pawnee wheat, when grown at 70°F under winter greenhouse conditions, is the best for identifying the 5 strains. Comparative tests based on dry weight of mature plants and field trials based on yield showed the yellow and leaf rolling strains to be significantly more virulent than the other 3. With all strains, Pawnee was susceptible, Concho was intermediate, and Triumph was tolerant.

Localized fungicide placement for control of garlic white rot. LEACH, L. D., AND W. S. SEYMAN. The limited zone of infection of *Scleratium cepivorum* on garlic suggested the possibility that a persistent fungicide at a suitable concentration might prevent infection within the protected zone. In December, 1953, several fungicides as wettable powders or solutions were sprayed into the furrow containing machine-planted garlic cloves by means of 2 nozzles arranged to distribute the fungicide as uniformly as possible from the bottom of the furrow to the surface of the soil. With a planting depth of 2 in. and furrow width of 2 in., 10 lb. of actual fungicide per acre furnished an average concentration of approximately 160 ppm (dry-soil weight basis). Captan at 6 or 12 lb./acre was ineffective. Calomel (HgCl) at 12 lb. gave fair protection, whereas $HgCl_2$ at 12 lb. or PCNB (pentachloronitrobenzene) at 25 lb./acre provided good protection. Similar trials in 1955 showed that PCNB sprayed as wettable powder or as emulsifiable concentrate at 15–25 lb./acre (240–400 ppm) or $HgCl_2$ at 10 lb. gave much better control than did calomel. Dusting the PCNB into the furrow gave protection comparable to spraying. Clove treatment with PCNB at 1% of clove weight reduced clove but not neck infection. Approximately 500 acres of garlic were treated commercially by this method during the 1956–57 season in central California.

The production of oligosaccharides by Verticillium albo-atrum. LE TOURNEAU, DUANE. A modified Czapek's mineral medium containing 3% sucrose was seeded with a mycelial suspension of *Verticillium albo-atrum* Reinke & Berth. At intervals, the medium was analyzed for sugars by paper chromatography. After 3–6 days, large quantities of sucrose and lesser quantities of glucose, fructose, and an unknown, A, were detected. Medium from cultures 6–10 days old contained sucrose, fructose, glucose, unknown A, appreciable quantities of a second unknown, B, and a trace of a third unknown, C. After 12–15 days, a trace of a material corresponding to raffinose was detected. All unknowns were less mobile than sucrose in a mixture of butanol, acetic acid, and water. Unknown C was less mobile than B, which in turn was less mobile than A. All unknowns gave a positive ketose test, were nonreducing, and yielded glucose and fructose on hydrolysis. The properties and mobilities of unknowns A, B, and C indicated that they were a trisaccharide, a tetrasaccharide, and a pentasaccharide, respectively, resulting from transfructosidase activity. Unknowns A and B were chromatographically similar to the trisaccharide and the tetrasaccharide, respectively, previously described by Pazur.

Survival of encysted eggs and larvae of the golden nematode to alternating temperatures. LEWIS, FREDA J., AND W. F. MAI. Sterilized soil infested with golden nematode cysts was maintained air dry and at seed-bed moisture in 1-pint containers. Both soils were shifted from 1 temperature to another (—5° and 75°F) at intervals of 1, 3, 7, 14, and 28 days for a period of 169 days. With some samples, a more gradual temperature change at time intervals of 3 and 14 days was included by inserting a 40°F treatment between —5° and 75°. At the end of the test period, viability of contents of cysts washed from the soil was tested by 1) counting the number of larvae emerging from cysts immersed in potato-root leachings and by 2) counting the number of immature golden nematode females developing on potato roots growing in soil to which cysts had been added. In dry soil, viability was similar following all treatments, including storage at constant temperatures of —5° and 75°F. In moist soil, greater losses in viability resulted from alternating temperatures than from constant temperatures; the loss in viability was correlated inversely with the length of the time period at a given temperature regardless of whether the change from 1 temperature to another was sudden or gradual. Some individuals survived all of the treatments.

Soil fumigation for control of the root lesion disease of walnuts. LOWNSBERY, B. F., AND S. A. SHER. Pressure-chisel machine applications of D-D (1,3-dichloropropene; 1,3-dichloropropane), Dowfume W-85 (1,2-dibromoethane), and Nemagon (1,2-dibromo-3-chloropropane) at walnut planting sites in 3 California orchards heavily infested with root lesion nematodes (*Pratylenchus vulnus*) have resulted in satisfactory growth of California black and Paradox hy-

brid walnut replants for 3 seasons following planting. Neither of these rootstocks has grown satisfactorily at untreated control sites. In San Joaquin County, growth of replants did not differ following preplanting treatments with D-D (60 gal./acre), Dowfume W-85 (15 gal./acre), or Nemagon (10 gal./acre); in all, growth was significantly better than in untreated controls. In Ventura County, D-D at 75 gal./acre resulted in significantly greater growth than did Nemagon at 10 gal./acre or Dowfume W-85 at 20 gal./acre; in all treatments, growth was significantly greater than that in untreated controls. Duration of benefits of preplanting fumigation is uncertain because soil populations of root lesion nematodes returned to pretreatment levels in 2 seasons. A second treatment of some of the established replants with Nemagon at 5 gal./acre at the start of the third season returned soil nematode density to a low level but produced no additional growth response.

Absorption and translocation of streptomycin by hops. MAIER, C. R., AND C. E. HORNER. Streptomycin sulfate and streptomycin nitrate (as Agristrep and Phytomycin, respectively) were readily absorbed and translocated by hop plants when applied in lanolin paste to the first node of stems. Both materials were assayed for inhibition of a streptomycin-sensitive strain of *Bacillus subtilis* by the filter-paper disk method; known concentrations of streptomycin in hop juice were used. No significant differences between the 2 materials were indicated by growth inhibition of the test organism. The regression line from the inhibition curve was used as a standard to determine streptomycin concentration in juice expressed from treated plants. Leaves 4–10 cm above the first node contained 76 ppm streptomycin sulfate and 81 ppm streptomycin nitrate 30 minutes after application. Streptomycin sulfate attained a mean concentration in the plants of 46 ppm after 24 hours, whereas streptomycin nitrate reached a mean concentration of 37 ppm after 15 hours. Both declined to 2.5 ppm after 72 hours but persisted at a concentration of 1.2 ppm after 12 days.

Reversal of virus-caused stunting by gibberellic acid. MARAMOROSCH, KARL. Gibberellic acid at 100 ppm was sprayed 3 times, at weekly intervals, on corn plants that had severe symptoms of corn stunt, on aster plants with aster yellows, and on crimson clover stunted by wound tumor virus. The treated plants resumed growth, whereas diseased controls remained stunted. The internodes of yellowed asters and stunted corn elongated to twice the original length, whereas the clover petioles increased 3 times in length and assumed an erect position during the 3-week period. In corn, the reversal of stunting became apparent after 48 hours, but in asters and clover it was noticed only after 5 days. Although stunting could be overcome to a considerable degree by 3 applications of gibberellic acid, diseased plants retained other symptoms of virus infection.

Determination of individual fungus spore volumes and their size distribution. MCCALLAN, S. E. A. To determine fungicide dose and other reactions involving spores on a fresh-weight basis it is necessary to know mean spore volumes. Densities of spores are about 1.1. By means of calculations based on the characteristic shape, e.g. a prolate spheroid, the mean spore volume in cubic microns was obtained for conidia (usually 100) of each of 20 species as follows: *Uromyces caryophyllinus* 15600; *Alternaria solani*, 9960; *Stemphylium sarcinaeforme*, 8680; *Erysiphe polygoni*, 7550; *Puccinia antirrhini*, 6180; *Uromyces phaseoli*, 6110; *Phytophthora infestans*, 5360; *Venturia inaequalis*, 616; *Botrytis cinerea*, 556; *Venturia pyrina*, 546; *Monilinia fructicola*, 523; *Alternaria oleracea*, 497; *Rhizopus nigricans*, 323; *Pestalotia stellata*, 320; *Glomerella cingulata*, 292; *Neurospora sitophila*, 283; *Neurospora crassa*, 99.3; *Aspergillus niger*, 29.2; *Myrothecium verrucaria*, 26.8; and *Cephalosporium acremonium*, 5.06. Size distribution, as determined by χ^2 and t tests for symmetry and kurtosis, was normal for 5 species, log-normal for 10, and intermediate for 5; that for small-spored species tends to be log-normal. Stability of mean spore volume is constant for some species but varies with others, depending on such things as time in culture and the particular lot collected. Different mechanical methods of collecting conidia of *M. fructicola* gave no differences in mean spore volumes.

Spore germination and cytology of Tilletia scrobiculata. MEINERS, J. P. Germination characteristics unique for the genus *Tilletia* were exhibited by spores of *T. scrobiculata* G. W. Fischer from leaves of *Poa secunda* when plated on soil-extract agar and incubated at 5° C for approximately 1 month. Either 2 or 3 (occasionally 4) primary sporidia were produced on a promycelium, in contrast to the relatively large numbers produced by other species. These sporidia are similar in morphology to those of *T. caries* but are approximately twice as large, ranging from 100 to 140 μ in length. They do not fuse and usually germinate from the base of the sporidium to form what appears to be an infection hypha, which elongates indefinitely. No secondary sporidia have been observed, and the primary sporidia do not grow in culture. Cytological studies reveal that the promycelium usually contains 8 nuclei before migration of the nuclei into the primary sporidia. Each primary sporidium may contain from 2 to 6 nuclei, the usual number being 4. The multinucleate nature of the primary sporidia and the lack of fusions between these sporidia suggest that this species is homothallic.

Compatibility relationships in species of Tilletia. MEINERS, J. P., AND J. T. WALDHER. Intraspecific and interspecific matings between pairs of primary sporidia of 5 species of *Tilletia* (*T. bromi-tectorum*, *T. caries*, *T. foetida*, *T. fusca*, and *T. guyotiana*) were made in culture. In 4 of the species, fusions occurred in approximately 50% of the matings between sporidia from the same spore and sporidia from different spores of the same species. Thus sporidial fusion within a species usually is controlled by a single factor with 2 alleles. In the first 3 species listed, production of infection hyphae was likewise controlled by a single factor. In the other 2 species, the percentage of infection hyphae produced was much lower than in the other 3, suggesting the presence of sterility factors for these species. In most of the interspecific matings, more than 80% of the pairs fused, indicating the possible presence of multiple alleles governing fusion between species. In contrast, infection hyphae were produced from approximately 50% of the matings; therefore, this process appears to be controlled by 1 allelomorphic pair. From these results, it appears that factors for compatibility may be distinct from those governing the production of infection hyphae.

Control of potato scab by a scab-suppressing factor in certain soils. MENZIES, J. D. Certain soils of the Yakima Valley, Washington, have a suppressing effect on potato scab that does not appear to be related to soil pH or to gross chemical or physical differences. Pot experiments conducted over a 4-year period have shown that incidence of scab in these soils does not increase with continuous potato cropping even when the soils are artificially infested with a virulent strain of the pathogen. This suppressing effect is entirely destroyed by pasteurization of the soil before inoculation. A striking reduction in the incidence of scab in a heavily infested soil was obtained by adding 1% by weight of scab-suppressing soil plus 1% by weight of chopped alfalfa hay. Addition of the soil alone was not effective at 10%, but scab was controlled completely when the rate was increased to 50%. Alfalfa hay alone usually increased the amount of scab. Beet pulp or wheat straw were ineffective when used in place of alfalfa. These data point to a microbial scab-controlling factor in these soils that can be established in other soils when introduced along with a suitable organic food source.

An undescribed ascomycete parasitic on the roots of potato. MENZIES, J. D., C. G. SHAW, AND C. C. GORDON. A superficial blackening of the fibrous root system of potato

(*Solanum tuberosum* L.) has been observed annually since 1944 in a series of crop-rotation plots at the Irrigation Experiment Station, Prosser, Washington. The condition, referred to as "black root," is the result of parasitism of the outer layers of the roots by an apparently undescribed member of the Meliolales. No disease symptoms other than the blackening of roots have been definitely associated with infection. Egg plant (*S. melongena* L.) also becomes infected when grown in the infested soil, but no other hosts are known. Attempts to grow the fungus on artificial media and to germinate the ascospores have been unsuccessful. The fungus produces a dense reticulum of dark hyphae (3.4–4.2 μ diameter) on the root surface. This mycelium bears lateral 2-celled hyphopodia from which dark, knot-like haustoria develop in the epidermal cells, giving the black appearance to the roots. Perithecia are superficial, black, flask-shaped, and ostiolate (200–310 $\times$ 105–140 μ), containing 1 to several ephemeral asci. Ascospores are brown and ovate (24–31 $\times$ 14–20 μ). They are 3-celled, the central cell much the largest, and are enclosed in a thin brittle sheath through which the apices of the spores protrude.

The relation of peach ring spot virus to sour cherry yellows, prune dwarf, and peach stunt. MILBRATH, J. A. Peach ring spot virus has been closely associated with sour cherry yellows, peach stunt, and prune dwarf. Attempts to separate distinct viruses as the causal agent for each disease have failed; therefore, the assumption that they might be caused by distinct strains of the ring spot virus was investigated. From 12 virus cultures that caused prune dwarf symptoms, 4 were selected because they appeared to contain 2 virus strains competing for dominance. From single buds selected from prune branches with normal foliage and from branches with prune dwarf foliage, 2 strains were isolated through peach to White Scallop squash. The isolate from the normal foliage caused a severe mottle and necrosis of the first leaf and a necrosis of the growing point on squash, whereas the one from the prune dwarf foliage caused stunting and a stipple mottle of the foliage. This distinctive correlation of symptom types in 4 different trials is interpreted as evidence that the virus causing prune dwarf is a specific strain of peach ring spot virus. This virus has now been returned to Mahaleb, and further testing of this relationship will be possible. Other studies indicated that peach stunt and sour cherry yellows also are caused by specific strains of peach ring spot virus.

An Alternaria leaf spot of Schefflera actinophylla. MILLER, H. N. A previously unreported, severe, leaf spot disease of Schefflera has been observed in Florida nurseries for several years. Leaf lesions are circular brown necrotic areas up to 35 mm in diameter. Elongated lesions may occur on the petioles, and infected leaves drop from the plant. A species of *Alternia* has been proved to be the cause of the disease. Conidia are dark brown, measure 70–185 $\times$ 18–45μ, and have 8–15 transverse septa and numerous longitudinal septa. The conidia are deeply constricted at the cell walls. They terminate in a hyaline septate beak ⅓ the length of the conidium. In greenhouse inoculations, the fungus did not infect potato, tomato, Acanthopanax, or Hedera. Because it possesses distinctive morphological and pathological characteristics, the organism is being described elsewhere as a new species.

Quantitative chlorophyll differences in peach leaves associated with infection by the phony peach virus. MILLIKAN, D. F. Chlorophyll components extracted from lyophilyzed peach leaves collected from diseased and disease-free Snyder Elberta trees indicated that those infected with the phony peach virus contained approximately 40% more chlorophyll pigments than did healthy leaves. Two samples of infected material contained 4.44 and 4.51 mg/g dry weight compared with 3.18 mg/g in the disease-free control. The ratio of chlorophyll *a* to chlorophyll *b* approached unity in all treatments, indicating that the change with respect to the green pigment was quantitative and not qualitative. Spectra of the cartenoid pigments indicated slight but hardly significant differences in total content. The absorption in the visible spectrum at 444 mμ and at 418 mμ suggested qualitative differences. These differences appear to lie in the xanthophyll fraction, but additional studies are needed to clarify this point.

Growth and leaf analysis of one-year-old apple trees grown in sand culture at different levels of calcium and potassium, with and without boron. MILLIKAN, D. F., E. E. PICKETT, AND W. J. UPCHURCH. Mealses-affected trees in Missouri respond to applications of boron yet rarely show fruit symptoms. Tests showed no difference in the boron content of soil around a healthy and that of soil around a diseased tree, but there was a high K/Ca ratio in the latter. Thirty-two scions from a diseased source and 32 from a healthy source were bench grafted and planted in sand as randomized paired samples; there were 4 replicates with a total of 16 trees within each. Trees were fed twice weekly: treatments consisted of a modified Hoagland's solution with a K/Ca ratio of 195/210 and one with a ratio of 540/30, both with and without boron. Growth measurements were made every other week and leaves analyzed at the end of the season. Growth for the minus-B treatments essentially ceased after the first month. Trees fed with the high-K solution grew only about 50% as much as those fed the normal solution, with or without B. Both B and K influenced Ca uptake. In the high-K$\pm$B treatments, the K/Ca ratio in leaves was approximately 12, whereas leaves on trees given the normal treatment ($\pm$B) showed a ratio of about 3. Trees on the high-K treatment contained approximately 15% more B than did those grown on normal solutions. The addition of B to the normal solution resulted in about 60% increase in uptake of Ca. This was also noted in the high-K treatment but was less pronounced.

Investigations on the curly dwarf virus disease of globe artichoke. MORTON, DONALD J. Electron micrographs of sap from curly-dwarf-affected artichoke plants (*Cynara scolymus* L.) have shown flexuous rods with mean lengths of 581.8mμ (S.D.=19.5) and mean widths of 15.4 mμ (S.D. =3.0). Similar rods were found in other hosts infected with the virus but not in healthy plants. Properties of the virus were determined to be as follows: thermal inactivation range, 55°–60° C; aging *in vitro* (19°C), 2–3 days; and dilution end point, 10^3–10^5, depending on virus source. Disease symptoms and percentages of artichoke plants infected were greatest at 13° and 19° C and absent at 30°C. The host range has been extended to include sunflower (*Helianthus annuus* L.), which develops chlorotic lesions with light-colored necrotic margins, and bachelor's-button (*Centaurea cyanus* L.), which develops necrosis of veins and twisting of shoots. Zinnia (*Zinnia elegans* Jacq.), previously reported as a systemic host, develops local lesions on all leaves at 13° and 19° C within 7–10 days following inoculation. Lesions on zinnia are necrotic with dark borders. Zinnia is a reliable indicator host for this virus.

Inheritance of resistance to leaf blotch of wheat caused by Septoria tritici. NARVAEZ, IGNACIO, AND RALPH M. CALDWELL. *Septoria tritici* Rob. ex. Desm. was grown for 8–12 days in 2% malt-extract broth in shake culture. Inoculum containing conidia and mycelium was prepared from a mixture of equal parts of the culture and a 1% water solution of gelatin. Mycelial and conidial cultures gave heavy infection whereas purely conidial cultures gave light infection. Inoculum was atomized over test seedlings in the 3-leaf stage, which were incubated for 48–60 hours at 65°–75°F. Initial symptoms appeared after 10–12 days, and infection results were recorded 3–5 days later. Seedlings were classified in 5 reaction classes, from 0 (immune) to 4 (susceptible). F_1, F_2, F_3, and backcross progenies of Nabob (resistant) $\times$ Knox and Vermillion (susceptible) indicated resistance to be controlled by 2 independent, partially dominant genes of additive effect. F_1 and F_2 and backcross pro-

genies of Lerma 52 and P14 (resistant) × Lee and Mayo 54 (susceptible) indicated that resistance of each resistant variety is controlled by a single dominant gene.

The growth of Phytophthora species as influenced by para-aminobenzoic acid. NEWTON, W. The growth of *Phytophthora parasitica*, an isolate from tomato, and of *P. parasitica* var. *nicotianae*, an isolate from tobacco, was markedly inhibited by *p*-aminobenzoic acid at concentrations as low as 20 ppm in oatmeal-dextrose broth shake-cultures. The growth of *P. cactorum*, an isolate from a crown rot lesion in apples, was also strongly inhibited by 80 ppm but not by lower concentrations. In contrast, 100 ppm had little effect upon the growth of *P. infestans*, an isolate from potatoes.

A rough bark disease of sweet cherry in California caused by a graft-transmissible virus. NICHOLS, CARL W., AND R. L. MCCLAIN. Young Lambert cherry trees with severe rough bark and excessive gumming on the trunk and basal portions of the lower limbs were observed in 1954 in a southern California orchard. In September, 1954, budwood from 2 of these trees was placed on Gaume peach, Shiro-fugen flowering cherry, sweet cherry (Lambert, Napoleon, and Bing varieties), and sweet cherry (Black Tartarian type but not true Black Tartarian). Typical ring spot symptoms appeared on all inoculated trees. In the second and third summers after inoculation, rough bark and gumming symptoms were observed on the inoculated Lambert and Black Tartarian trees. No definite rough bark symptoms have been seen on the inoculated Napoleon and Bing trees or on the noninoculated check trees of each sweet cherry variety. The first rough bark symptoms on inoculated Lambert and Black Tartarian appeared as discrete blisters similar to those produced by the necrotic rusty mottle virus in California. These blisters appeared to coalesce and rupture, forming rough areas from which masses of gum were exuded. No foliage symptoms other than those of ring spot were observed on the originally infected Lambert trees or on the inoculated trees. The relationships between this disease and other stone fruit virus diseases are yet to be determined.

Heat inactivation of ringspot virus in some stone fruit hosts. NYLAND, GEORGE. Ringspot virus was inactivated in portions of trees of Stockton Morello sour cherry, sweet cherry, and Lovell peach seedlings after they were held for 17–24 days at 100°F air temperature and 85°F soil temperature. Up to 9 months after budding, Stockton Morello trees propagated from trees heated for 17 days have indexed negative for ringspot virus in the Shiro-fugen and cucumber tests. Unheated trees have consistently tested positive. In most cases, treated plants showed shock symptoms of ringspot in the new shoot growth about 3-4 weeks after latent or dormant buds began to grow. This permitted time for propagation before ringspot virus reinvasion occurred. Lovell seedlings known to be carrying ringspot virus in association with sour cherry yellows, prune dwarf, and cherry rugose mosaic viruses all indexed negative for ringspot after being heated for 24 and 32 days but not after being heated for only 17 days. In some cases, Lovell seedlings survived up to 43 days of exposure. It is suggested that in the field temporary inactivation or immobility of ringspot virus may occur and some current-season buds thereby may be free of ringspot virus. The following season the leaves developing from such buds would show shock symptoms when reinvasion occurs. This could explain the recurrent-type symptoms of ringspot such as tatterleaf or laceleaf. Evidence obtained from these experiments indicates that the same strain of ringspot virus may be more easily inactivated in cherry than in peach.

The dried fruit beetle disseminates spores of the peach brown rot fungus. OGAWA, JOSEPH M. The dried fruit beetle, *Carpophilus hemipterus*, was found to feed on brown-rot (caused by *Sclerotinia fructicola*) affected peach fruits in Sacramento Valley orchards. Dried fruit beetles collected in orchards were found to have been contaminated with spores of *S. fructicola* during their feeding on diseased fruits on either the tree or the ground. Laboratory and field trials showed that these beetles will not attack uninjured areas of mature fruits. Field evidence that the brown-rot disease originates in many cases in natural injuries, such as split pits and bruises, suggested the possibility that the beetles might disseminate spores of the pathogen to these avenues of infection. Contaminated beetles in caged trees were found to be attracted to natural or artificial wounds, and inoculation by this means was found to be responsible for production of disease. The practice of flood irrigation, which covers the insect-infested diseased fruits on the ground, is thought to be conducive to dissemination of the pathogen by inducing contaminated beetles to move into the tree to find new sources of food.

Relative efficiency of airplane and ground application of sprays in controlling almond shot-hole disease. O'REILLY, H. J. Airplane and conventional spray applications of ziram were made on almonds (*Prunus amygdalus* var. Drake) at 50% bloom and at petal fall. The objective was to compare their relative efficiencies, since no information is now available on aerial methods of tree fruit disease control in California. Airplane applications consisted of approximately 60 gal. of spray per acre and the ground applications of slightly over 300 gal. The first ground application was made simultaneously with the aerial application, whereas the second was applied 3 days prior to the corresponding aerial application. No rains, which might favor shot hole (caused by *Coryneum beijerinckii*) development, occurred during this 3-day period. Ninety per cent of the fruit on unsprayed trees and on those sprayed from the air was infected, whereas only 10% of that on trees given ground applications was infected.

Potassium and internal black spot of potato in California. OSWALD, JOHN W., AND OSCAR A. LORENZ. The occurrence of black spot of Russet Burbank potatoes in the Santa Maria and Salinas valleys appears to be associated with potassium nutrition. In a survey of 25 fields, black spot was found in 61.6% of the tubers from fields where vines contained 2–4% K (about 80 days after planting); the average severity was 1.12 (3, 2, and 1 represent severe, moderate, and mild black spot, respectively). Of the tubers from fields with vines analyzing 10–12% K, only 28.4% had black spot and the average severity rating was 0.39. Two test fields in which yield response to potash did not occur had vines containing 10.18 and 10.12% K at approximately 90 days; subsequently, 25.2 and 29.6%, respectively, of the tubers had black spot, and the severity ratings were 0.33 and 0.37, respectively. In 3 potash-deficient test fields, comparable-aged vines analyzed 0.70, 1.81, and 1.54% K and the subsequent percentages of black spot were 73.7, 84.2, and 74.5, respectively; the average severity ratings were 0.94, 1.50, and 1.21, respectively. In portions of these fields fertilized with 600 lb. potash per acre, the 90-day-old vines contained 3.58, 6.74, and 7.74% K, respectively, and the percentages of black spot were 51.8, 79.8, and 72.9, respectively. The data suggest that black spot is very mild (severity index < 0.5) only when the potassium level of vines at 80 days is at least 9.5–10%; 600-lb. applications of potash did not accomplish this in the above fields.

Studies concerning the ghost-spot disease of tomato. OWEN, JOHN H., AND JUAN B. FERRER. The tomato ghost-spot disease has become of increasing importance in the tomato-growing areas of Florida. The disease has previously been ascribed to *Botrytis cinerea* Pers.; however, it is not uncommon to find severe infestations of this fungus in tomato fields with little or no ghost spot present. Inoculation of numerous excised tomato fruits with conidia of *B. cinerea* always produced the small necrotic specks without halos. Inoculation of tomato fruits on plants grown in the greenhouse gave similar results, with the ghost-spot symptoms appearing on less than 1% of the inoculated fruits. When fruits on plants grown in outside plots were inoculated dur-

ing the cool night period and exposed to the bright sunlight and high temperatures the following day, ghost spot appeared on 57% of the inoculated fruits. Inoculated tomato fruits that were exposed to various light intensities, to ultraviolet rays, to low and high temperatures, or to combinations of these factors, however, always failed to develop typical ghost spots. Histological studies revealed that an early-stage necrotic speck consisted of a single necrotic cell, though later many cells were involved. Sections through young spots on inoculated fruits and on naturally infected fruits stained with lactophenol plus cotton blue usually showed a stainable fungus-like material in the necrotic speck. Attempts to isolate a fungus from the diseased areas always gave negative results.

Intracellular distribution of radioactive fungicides and metal ions in fungus spores. OWENS, ROBERT G., AND LAWRENCE P. MILLER. The intracellular distribution of C^{14}-labeled dichlone and glyodin and of Ag^{110}, Zn^{65}, Cd^{115}, Hg^{203} and Ce^{144} absorbed from aqueous solution by conidia of *Neurospora sitophila* and of *Aspergillus niger* was determined. After absorption and removal of external fungicide, conidia were disintegrated sonically. Cell walls were separated from the homogenate by centrifuging at 500 G for 5 minutes. The supernatant was partitioned into 3 fractions by centrifuging at 10,000 G for 30 minutes (fraction A) and again at 80,000 G for 1 hour (fraction B). The supernatant from the last centrifugation was called fraction C. Little if any of either fungicide was retained by the cell walls. Considerable amounts of both fungicides and of the metal ions accumulated in particulate fractions A and B. Various amounts, depending on the fungicide or ion, were contained in fraction C. About 75% of the total glyodin was associated with fraction B, and most of it could be extracted with acetone. About 75% of the total dichlone was found in fraction C; that associated with fractions A and B could not be extracted with fat solvents. A large portion of the metal ions was found in fractions A and B. The data show that dichlone, glyodin, and metal ions are absorbed by fungus spores and not merely adsorbed at the surface or in the spore wall.

A new slit-type continuous spore sampler. PADY, S. M. A sampler designed to determine spore loads, particularly of spores of rust fungi, takes hour-long samples continuously through a 24-hour period and deposits spores in 24 bands on a 1 × 3-in. slide. A feature of the sampler is the direct attachment of a squirrel-cage type blower (110 volt, 60 cycles, 50 cu. ft./minute capacity) to the sampling column. The silt assembly is 4 in. from the top half of the column, which rotates freely; the aperture (2 × 14 mm) is 2 mm from the slide. The blower and electric terminals are attached to the lower fixed half. The flow rate is 0.4 cu. ft. per minute. The timing mechanism consists of 2 electric motors (110 volts, 60 cycles, 2.5 watts, 1–60 rpm and 1 rpm) and relay switch. Once hourly the switch activates the second motor attached to the slide holder, moving the slide a short distance. A weather vane keeps the slit facing the wind, and a metal shield protects the slit opening. The blower and timing mechanism are mounted on a wooden frame and are protected from the rain. The sampler has performed satisfactorily under severe weather conditions. With the sampler, the number of spores per cu. ft. of air may be determined for any hour of the day.

Seasonal occurrence of leafhopper vectors of X-disease virus in sprayed and unsprayed peach blocks. PALMITER, D. H., AND J. A. ADAMS. Leafhoppers were collected on Tanglefoot-coated trapboards hung in peach trees at weekly intervals from May to November of 1955 and 1956. Sprays of DDT and malathion were applied at 10-day intervals to half of the orchard. *Scaphytopius actus* (Say) was the most abundant species collected, with a total of 348 recorded in 1955 and 283 in 1956. Collections of this species started the second week of June and reached a peak in early July. Few were found from mid-July to mid-August. A late brood reached its peak in September, but individuals were still present in November. Specimens of *Colladonus clitellarius* (Say) were collected during the same periods as was *S. acutus* but in smaller numbers. In 1955 a total of 7 sprays reduced the number of *S. acutus* from 68 to 41 (between June 6 and August 16), but in 1956 the reduction was slight (69 to 62). The small size of the sprayed block (¾ acre) and the presence of a grass and clover ground cover may have been factors in the poor control of leafhoppers in this experiment. The presence of vectors from June to November, however, seems to preclude the use of insecticides as an economical means of preventing the spread of X-disease.

Growth in vitro at various temperatures and acidities of tissues isolated from insect galls and from normal stems of elm, grape, poplar, and willow. PELET, FRANCIS, AND ALBERT C. HILDEBRANDT. Tissue cultures from insect galls and from normal stems were compared on synthetic media supplemented with coconut milk (CM) and naphthaleneacetic acid (NAA) (Medium I) and with CM, NAA, and 2,4-dichlorophenoxyacetic acid (Medium II) at temperatures from 16° to 36° C, at 4° intervals, and at 26° over a range extending from pH 3.5 to 8. On Medium I, tissues of *Populus deltoides* grew best at 28° and 32° and at pH 7.5; the normal stem tissue grew faster than the Pemphigus petiolar gall tissue. All tissues of *Salix nigra* grew best from 20° to 36°; normal stem tissue grew best on II at pH 6, and Pontania leaf gall tissue and the tissue of a twig gall of unknown origin grew best on I at pH 4.5. Normal stem tissue of *Ulmus americana* grew slowly but best at pH 4 and between 24° and 36°; the elm coxcomb gall tissue behaved similarly except that it grew better than did the normal tissue at 28° and 32°. In tests with tissues of *Vitis vulpina*, normal stem tissue grew best on I at pH 4 and at 36°, Cedidomyia cylindrical petiolar gall tissue grew best on I at pH 4.5 and at 32°, and Phylloxera leaf gall tissue grew best on II at pH 7 and at 28°. Tissues of all species generally were inhibited above pH 7 on I and below pH 4 on II. Considerable variability appeared within the individual isolates.

Comparative studies of the fungus causing the dead-arm disease of grapes. PINE, THOMAS S. Isolates of the dead-arm fungus from Canada, South Africa, New York, and California were found to be identical in morphology of the fruiting structure and in spore size and shape when grown on synthetic media and on sterilized grape wood. No grape variety has shown resistance to the fungus. Morphological comparisons of the 4 isolates have shown that they are the same fungus, *Phomopsis viticola* Sacc. Both pycnidiospores (α) and scolecospores (β) were produced on sterilized grape wood. In culture, increasing the carbon source increased the size of the oil bodies in the α-spores so as to distort the spores. On basal medium containing 32–64 g dextrose/liter, α-spores formed but remained attached to the sporophores and were released as a unit. On basal medium containing 128 g dextrose/liter, β-spores were produced in much larger quantities than were α-spores. β-Spores have produced germ tubes on water agar, potato-dextrose agar, and nutrient agar. Infection, however, has been observed to be effected only by α-spores. Penetration of the host occurs through leaf and shoot stomates, or through wounds, but this occurs only after 4-8 hours in continuous moisture.

Effect of thiouracil on $C^{14}O_2$ fixation in healthy tobacco plants and in tobacco plants infected with cucumber mosaic virus. PORTER, CLARK A., AND LEONARD H. WEINSTEIN. Vamorr 48 tobacco plants systemically infected with cucumber mosaic virus and comparable noninfected plants grown in nutrient cultures containing 0 and 5 ppm of thiouracil were examined for their capacity to incorporate $C^{14}O_2$ into certain metabolites. Thiouracil treatment was initiated 48 hours prior to inoculation with virus and was continued for a total of 286 hours. At this time, leaves of the plants were injected with $Na_2C^{14}O_3$; they were harvested 2 hours later. As compared with the control, thiouracil treatment alone increased fixation of $C^{14}O_2$ into free amino acids, sucrose,

glucose, fructose, malic acid, and citric acid, whereas it decreased the amount incorporated into protein. Virus infection alone increased the fixation of $C^{14}O_2$ into sucrose, glucose, fructose, and citric acid, whereas it decreased the incorporation into free amino acids, protein, and malic acid. The combination of thiouracil treatment and virus infection resulted in a pattern of $C^{14}O_2$ fixation similar to that produced by thiouracil alone.

Effect of soil temperature on the time of infection of Red Bobs wheat by Tilletia caries. PURDY, LAURENCE H., AND EDGAR L. KENDRICK. Wheat seed inoculated with spores of *T. caries* (DC.) Tul. was planted in sand maintained at the following temperatures: 5°, 10°, 15°, and 20°C. Seedlings were removed at 24-hour intervals, washed in running water, and divided into 2 groups. One group was transplanted without further treatment into soil in greenhouse benches; the other group was surface sterilized, prior to planting in the greenhouse, by soaking in a 1:1000 water dilution of Panogen 15 (2.2% cyano (methylmercuri) guanidine) for 2 minutes to prevent further infections by spores carried on the seed. All plants were grown to maturity, and percentages of bunt were determined. Maximum percentages (63–100%) of bunted plants developed at 20°, 15°, and 10°C after 7, 8, and 10 days, respectively. Only low percentages (12–14%) occurred at 5° after 20 days' incubation. The number of days required for maximum development of bunt decreased as the temperature was increased to 20°.

Shot hole of Catalina cherry. RAABE, ROBERT D. In the spring of 1956, at Fresno, California, a planting of Catalina cherry, *Prunus lyonii* Sarg., was found to be severely affected by a shot hole disease. Following this outbreak, the disease was found in several other locations in California. The fungus associated with the disease was identified as *Coryneum beijerinckii* Oud., the fungus causing the shot hole disease of stone fruits. The fungus from Catalina cherry was found to produce lighter-colored, slower-growing colonies than did an isolate of *C. beijerinckii* from peach when both were grown on potato-dextrose agar. On plant tissue sterilized with propylene oxide, the conidia from the peach cultures were approximately 10 μ shorter and 1.5 μ wider than the conidia from the Catalina cherry cultures. In cross-inoculation tests, the isolates from both peach and Catalina cherry infected both peach and Catalina cherry. It is believed that this is the first report of this fungus on Catalina cherry.

Fusarium wilt of Hebe buxifolia. RAABE, ROBERT D. *Hebe buxifolia* Cockayne & Allan, sometimes called *Veronica buxifolia* Benth., is a small shrub, frequently used as an edging in California gardens. A serious, previously unreported disease that results in the death of infected plants was found recently in a nursery in the San Francisco Bay area. A form of *Fusarium oxysporum* (Schl.) Snyd. & Hans. was isolated from the diseased plants. Rooted cuttings of healthy plants, after being dipped in a spore suspension of the fungus and planted in pots, began showing symptoms after 10 days and were killed within 3 weeks. The symptoms appeared as a progressive chlorosis, followed by a browning of the leaves beginning at the base of the stems. Frequently, the plants died before these symptoms reached the tops of the plants. The stem tips of affected plants curled over, giving the appearance of wilting. Herbaceous members of the Scrophulariaceae, including plants in the genera *Veronica, Penstemon, Digitalis, Antirrhinum,* and *Mimulus,* did not become infected following inoculation. This is the first record of a Fusarium vascular wilt in the genus *Hebe.* Since the fungus appears to be a previously undescribed form of *F. oxysporum* pathogenic to *Hebe buxifolia,* it is named *Fusarium oxysporum* f. *hebae* n.f.

A local-lesion test for potato virus A in the presence of potato virus X. RAYMER, W. B., AND J. A. MILBRATH. Local lesions have been reported in various host plants after inoculation with potato virus A. These same hosts gave similar local lesions when inoculated with potato virus X. Since most commercial potato varieties in North America are uniformly infected with virus X, a host that would react only to virus A would be valuable for rapid diagnostic tests. Köhler's "A6" virus-free clone of *Solanum demissum* × *S. tuberosum* var. *aquila* gave necrotic local lesions when inoculated with either virus X or virus A or with both viruses simultaneously. Although the respective lesions were slightly different, those due to virus A alone could not be distinguished satisfactorily when virus X was present. We inoculated "A6" plants with virus X and allowed them to become systemically infected. The resulting tubers produced plants that formed no lesions when inoculated with virus X, but virus A alone or combined with virus X readily induced the lesions characteristic of virus A. This protection against lesion formation by virus X made these plants useful as a diagnostic host for virus A, even when virus X was present in the inoculum.

N-Acetylanthranilic acid derivatives for control of cucumber powdery mildew. RICH, SAUL, AND JAMES G. HORSFALL. *N*-Acetylanthranilic acid (Cr 491), copper *N*-acetylanthranilate (Cr 495), copper *N*-acetyl-5-chloroanthranilate (Cr 977), and *N*-(chloroacetyl) anthranilic acid (Cr 1142), each at 1 and 2 lb. of active ingredient per 100 gal., were compared with Karathane (22.5% 2-(1-methyl heptyl)-4,6-dinitrophenyl crotonate) at 1 lb. of formulation per 100 gal. for control of cucumber powdery mildew in the greenhouse. The experimental compounds, obtained from Rohm and Haas Company as technical materials, were suspended in water without added wetting agents. The plants were sprayed, allowed to dry, and then inoculated. Karathane, the high concentration of Cr 1142, and both concentrations of Cr 495 and Cr 977 significantly reduced disease development. Only Karathane and the high concentration of Cr 977 completely protected sprayed leaves against mildew. Only Karathane produced any spray injury. Because disease developed on some stems in all treatments except Karathane and because there was no external redistribution, it is likely that Karathane has some systemic action against powdery mildew of cucumbers. The protection of cucumbers against powdery mildew by Cr 495 confirms earlier results with this compound and with other metal salts of *N*-acetylanthranilic acid for the control of powdery mildew of roses and snapdragons.

The influence of light and temperature on the development of corn rust. RIKSH, SYAMANANDA, AND J. G. DICKSON. Experiments were designed to study the effects of temperature and light upon the development of *Puccinia sorghi* Schw. in selected corn inbreds. Rust response was influenced more by temperature than by light. Plants grown under additional light generally showed increased necrosis around uredia than did plants grown under natural light conditions. High temperature (33° C) and low light intensity prevented further development of rust after the pathogen was established in certain inbred corn lines. Full expression of seedling resistance was prevented in most corn lines grown at 28° C, although susceptibility was decreased in some lines at the higher temperatures. Some inbred corn lines were susceptible within 1 temperature range and resistant within another. The incubation period increased as the temperature was decreased. The temperature and light conditions used prior to inoculation also influenced the type of uredia and the amount of sporulation. Some inbred corn lines susceptible in the seedling stage were less susceptible or resistant when older. Resistance in these older plants was not expressed at high temperatures.

Fumigation with ammonia gas for control of Penicillium decay of citrus fruits. ROISTACHER, CHESTER N., L. J. KLOTZ, AND I. L. EAKS. Exposure of citrus fruit to an atmosphere containing 50–300 ppm ammonia gas for 5–10 hours has repeatedly given control of the blue-green mold decay caused by *Penicillium italicum* and *P. digitatum.* The concentration of ammonia gas in the air was determined by

nesslerizing aspirated samples. A formula for predicting decay control without fruit injury was based on the product of gas concentration in the air surrounding the fruit multiplied by the duration of exposure (ppm NH_3 × time in hours = ammonia index). Good control has been realized with an ammonia index of 500–3000; injury of the fruit occurred above 3000. Commercial gas treatments conducted since January, 1956, on both navel and Valencia oranges have shown the complete practicability of ammonia fumigation for control of the blue-green molds.

Patterns of ammonia release in cartons as related to control of decay of citrus fruits. ROISTACHER, CHESTER, N., L. J. KLOTZ, MARTIN J. KOLBEZEN, AND EDWIN A. STAGGS. In attempts to formulate efficient ammonia-releasing compounds for use in cartons, gaseous emission patterns of such compounds were studied. These emission patterns were directly compared with the control of decay on oranges wounded and inoculated with *Penicillium digitatum* and *P. italicum*. Decay control under simulated refrigerator-car conditions was proportional to the quantity of ammonia emitted from pellets or sheets of various formulations. At controlled temperatures, emission of ammonia from pellets and sheets was significantly curtailed at 32°F as compared to emission at 50° or 68° F; again, control was proportional to the quantity of gas released. In 1 experiment conducted under refrigerator-car conditions, ammonia gas was injected into 10 × 11 × 16½-in. cartons, each containing 100 wounded and inoculated oranges. Injections of ⅛, ¼, ½, or ¾ g per carton were made on all combinations of the first, fifth, and/or ninth day after inoculation. Injections made the first day were not as effective as those made later. The best control of decay and the greatest reduction of spore viability resulted from 2 or 3 injections of ½ or ¾ g per carton; there was no injury to the fruit.

Pathological respiration in Fusarium-infected tomato plants. SCHEFFER, R. P., AND R. P. COLLINS. Respiration in leaves of tomato plants was stimulated shortly after infection with *Fusarium oxysporum* f. *lycopersici*. Nine to 14 days later, oxygen uptake by diseased leaves was as much as twice that of healthy controls. Ethylene and fusarinic acid were considered as possible causes of stimulated respiration. Leaves were treated with various mixtures of ethylene and air or with various concentrations of ethylene in solution, but in no case was respiration stimulated. Fusarinic acid inhibited leaf respiration in concentrations above 0.0005 M; it had no effect in more dilute solutions. Both ethylene and fusarinic acid were eliminated as causes of pathological respiration. Treatment with 2,4-dinitrophenol stimulated oxygen uptake by leaves from infected plants to the same degree that it stimulated oxygen uptake in healthy leaves. The ratio of CO_2 production in nitrogen to CO_2 production in air did not differ materially in diseased and healthy leaves. It was tentatively concluded that pathological respiration does not depend on uncoupling reactions or on interference with the Pasteur effect. Leaf tissues became increasingly less sensitive to malonate as the disease developed.

Some host-range studies of peach yellow bud mosaic virus. SCHLOCKER, ARCHIE, H. KEITH WAGNON, AND JAMES R. BREECE. Yellow bud mosaic (YBM) virus was recovered from a Wickson plum (*Prunus salicina* × *P. simonii*) tree growing on plum rootstock in a California planting of peaches and plums wherein YBM incidence in the peaches was high. Symptoms suggestive of YBM were present in the plum. In 1952, buds from the plum were placed in 4 Manchu cherry (*P. tomentosa*) plants. In 1953, the 4 plants showed severe symptoms strongly suggestive of YBM. Buds from one were placed in 2 trees each of the following top and rootstock combinations: Elberta peach/Lovell peach, Wickson plum/myrobalan 29c plum, Blenheim apricot/Lovell peach, and Bing sweet cherry/mahaleb cherry. In 1954, the peach and apricot had symptoms typical of YBM and the Wickson plum had leaf symptoms similar to those on the original Wickson source. No symptoms suggestive of YBM have appeared on the Bing cherry after 4 years; attempts in 2 separate years to recover the virus into peach failed. When buds from Elephant Heart and Kelsey (both *P. salicina*) and myrobalan plums growing on YBM-affected peach rootstock were placed in healthy Elberta peach/Lovell peach, no YBM symptoms developed in the Elberta peach after 2 years. Only the Kelsey plum had shown symptoms suggestive of YBM. Symptoms suggesting YBM were seen on old Gaviota plums (*P. salicina* × *P. americana* × ?) growing on peach rootstock adjacent to YBM-affected peach trees. Recovery of YBM virus from them has not yet been attempted.

Microclimates and their significance in the development of powdery mildew of lettuce. SCHNATHORST, W. C. Wet and dry thermocouples connected to a Leeds and Northrup potentiometer were used to measure relative humidity (RH) in small spaces. RH readings increased between 5 and 10% as the thermocouples were lowered from a height of 1 m to a point 2 cm above the surface of the soil in a wet field of lettuce, whereas there was no significant gradient in RH over a dry field of lettuce. Measurements of gradients in RH were made above lettuce leaves in the laboratory and over distilled water as a check on the accuracy of the method. The RH was 100% at the surface of the water but only 67% at the leaf surface in a room where the RH was 55%. Percentage of germination of conidia of the lettuce powdery mildew fungus (*Erysiphe cichoracearum* DC.), a biological measurement of RH at the leaf surface, indicated that the RH at such surfaces was less than 75% (germination below 75% RH is constantly low at 2%). It is estimated from meteorological data that microclimates add at most 2 hours of each day to the period favorable for germination of conidia (95–99% RH at 25°C). Although microclimates may influence disease development to some extent, it appears that this disease is influenced mainly by the macroclimate.

An osmotic mechanism for resistance to powdery mildew in lettuce and peach. SCHNATHORST, W. C., AND A. R. WEINHOLD. Resistance in lettuce to the lettuce strain of *Erysiphe cichoracearum* is negatively correlated with the combined thickness of the cuticle and epidermal wall. Resistant lettuce leaves became susceptible when placed in detached-leaf culture and when etiolated for short periods. These findings indicate that resistance is physiological. Differences in osmotic pressure appear to be a possible underlying mechanism of resistance (high osmotic pressures reflecting resistance in the host), and this mechanism may be responsible for the presence of disease gradients on lettuce plants, for resistance of young leaves of field-susceptible varieties, and for resistance of all leaves of field-resistant varieties. Resistance in the Paloro variety of peach to *Sphaerotheca pannosa* also appears to be physiological. There is a disease gradient on leaves of different ages: young peach leaves are susceptible whereas older leaves are resistant (reversed from the situation in lettuce). Resistance is directly correlated with the osmotic value of the cell sap. Sucrose solutions with osmotic values equal to those of resistant lettuce (17 atmospheres) and peach (18 atmospheres) leaves inhibited water uptake by conidia of the respective pathogens, as indicated by inhibition of germination.

Chromatic parenchyma cells in tristeza-diseased Citrus. SCHNEIDER, HENRY. The first response of Mexican lime seedlings to graft inoculations with tristeza virus was an increase in the thickness of the cytoplasmic layer and an intensification in staining in the elongated parenchyma cells adjacent to sieve tubes. Cells so affected are hereafter called chromatic cells. The central vacuole became reduced from a major to a minor portion of the cell and was sometimes divided into several small ones. Within the chromatic cells,

irregular masses and strands of material stained more densely than did the cytoplasmic matrix in which they were embedded. The dense material eventually divided into needle-like bodies. Sometimes, these masses and needles stained with Giemsa stain as do viruses. The chromatic condition sometimes spreads from the parenchyma cells adjacent to sieve tubes to nearby phloem parenchyma cells and even to other tissues, e.g. from the protophloem to the differentiating cortex and to the interfascicular parenchyma cells but rarely from primary phloem to the primary xylem. When isodiametric cells were affected, the normally thin layer of cytoplasm only increased slightly in thickness. Chromatic cells were also found in Meyer lemon trees, Lisbon lemon seedlings with seedling yellows, and above (but not below) the bud union of sweet orange trees on sour orange rootstock.

Anatomical response of some Citrus species to tristeza virus. SCHNEIDER, HENRY. In Mexican lime plants, lesions were initiated from groups of chromatic cells described in another paper. Hypertrophy of partially chromatic cells and adjacent nonchromatic cells occurred, and some chromatic cells were crushed. This was followed by hyperplasia and by a failure of normal differentiation of tissues. For instance, in affected areas of the protophloem there was hypertrophy, hyperplasia, crushing, and failure of primary phloem fiber differentiation. When secondary tissues were present, localized areas of the cambium and of adjacent xylem and phloem mother cells sometimes were affected. As a result, a disorganized mass of soft tissue was formed in place of xylem and phloem, and pitting of the wood resulted. Stem pitting and vein clearing both result from the formation of chromatic cells followed by hypertrophy, hyperplasia, and disorganization. The sieve-tube necrosis and subsequent disturbances produced in the sour orange rootstocks of tristeza-affected sweet orange trees is entirely different from the injury here described in the lime seedlings. Therefore the rootstock injury probably is caused by the tristeza-diseased sweet orange scion and not by the virus.

Stability and movement studies on the new experimental nematocide 3,4-dichlorotetrahydrothiophene-1,1-dioxide in soil. SCHULDT, PAUL H., H. P. BURCHFIELD, AND HENRY BLUESTONE. Stability and movement of 3,4-dichlorotetrahydrothiophene-1,1-dioxide (PRD) in soil was determined quantitatively by a colorimetric procedure that depends on a reaction with pyridine and alkali. The half-life of PRD in soil is concentration dependent. In moist soil in a closed jar at an initial concentration of 100 μg/g it had a half-life at pH 6.5 of 20 days at 32°C, 45 days at 22°C, and 115 days at 10° C. PRD persisted in dry soil 3 times as long as in moist soil. It is sensitive to alkali; after 53 days in moist soil at 22°, 98% of the chemical was lost at pH 8.3, 90% at pH 7.4, and 62% at pH 6.6. As long as PRD remains in soil, it moves downward as a result of rainfall and upward during drying periods. Initially it moves downward in a vertical band 3–4 in. wide, similar to movement in a chromatographic column, but with additional rainfall this band widens and PRD tends to become uniformly distributed vertically. In field applications made in the fall, distribution followed this pattern irrespective of whether PRD was placed on the soil surface or worked into the top 3 or 6 in. PRD penetrated to a depth of more than 15 in. after 6 in. of rain, and about 50% of the chemical was recovered after 178 days in soil at pH 5.

Extent of the endophytic system of dwarfmistletoe on Pinus ponderosa. SHEA, K. R. In each of 2 areas in southern Oregon, 2 branches of ponderosa pine with localized infections of dwarfmistletoe (*Arceuthobium campylopodum* f. *typicum* (Engelm.) Gill) were collected from each of 18 combinations of 3 branch diameters, 3 branch heights, and 2 distances from the tree bole to the proximal end of the external swelling associated with infection. Each branch was dissected and examined microscopically to determine the relation of the endophytic system to the associated swelling. Analyses of variance brought out the significant effect of an increase in branch diameter on the proximal extent of both the endophytic system and the corresponding swelling. Branch height and distance of the swelling from the tree bole in the 2 areas showed no consistent effect on the extent of either the endophytic system or the swelling length. Further analyses brought out highly significant, positive coefficients of correlation between the swelling and the corresponding endophytic system for both proximal and total lengths. The relationships discovered from this study suggest that pruning to remove dwarfmistletoe-infected branches can be done with a high degree of confidence when the proximal end of the external swelling associated with infection is more than 4 in. from the tree bole.

Variability among strains of noncultivated species of oats for reaction to races of the crown rust fungus. SIMONS, M. D. About 200 strains of oats, distributed among all the major noncultivated species of the genus *Avena*, were tested 4 times as seedlings for reaction to 8 races of *Puccinia coronata* Cda. var. *avenae* Fraser & Led. Forty-four strains showed at least some degree of resistance to at least 1 race in at least 1 test. When 3 reaction classes (resistant, intermediate or variable, and susceptible) were used, the 44 strains fell into 21 groups, indicating that at least that many different genetic systems for crown rust reaction were present. C.I. 3815 (*Avena strigosa* Schreb.) was highly resistant to all 8 races and to about 800 additional isolates of various races, including races that parasitized the key differential varieties Saia, Glabrota, and Landhafer. Except for an intermediate reaction to a few races, P.I. 193958 (*A. abyssinica* Hochst.) was equally promising. C.I. 2524 and C.I. 2835 reacted like Glabrota, whereas C.I. 3436, C.D. 3820, and P.I. 194201 reacted like Saia. A few strains that were fully susceptible as seedlings were resistant as older plants in the field.

Nitrogen nutrition of squash and the multiplication of squash mosaic virus. SINGH, G. P., AND GLENN S. FOUND. Squash plants were grown at nitrogen concentrations of 21, 210, 630, and 1050 ppm, with nitrate as the sole source of nitrogen except at 1050 ppm, where it was supplied as both nitrate and ammonium nitrogen. Host growth was depressed at 630 ppm in comparison to 210 and 1050 ppm. Virus concentration, as measured by optical density on a sap-aliquot basis, generally increased with increase in nitrogen up to 630 ppm. Virus concentration did not reflect the depression in host growth at 630 ppm. In other experiments in which both nitrate and ammonium nitrogen were used to obtain levels of 630 and 1050 ppm, growth consistently increased with increase in nitrogen up to 630 ppm; at 1050 ppm, marked stunting occurred. Virus concentration generally followed the growth curve. No correlation between acidity of sap extracts and virus concentration was evident, but the pH of sap from infected plants at all levels of nitrogen was slightly lower than that of sap from noninoculated plants. The depressed growth at 630 ppm, with all nitrogen supplied by nitrate, is believed to be due to toxicity of sodium ions, which were in greater proportion in solutions at this level than in those containing 1050 ppm.

Studies in the disease resistance of Impatiens balsamina. SPROSTON, THOMAS, JR. *Impatiens balsamina* is relatively free of fungus diseases despite the fact that spores of various fungi, such as *Alternaria tenuis*, *Botrytis allii*, and *Monilinia fructicola*, germinate well (66–93%) on its leaf surfaces and penetrate into epidermal cells. A dark stain appears in the invaded cells, but no further disease develops. The dark stain in the invaded cells suggests the presence of free polyphenols. Several polyphenolic compounds—2,3-dimethoxy- and 2,methoxy-1,4-naphthoquinone, kaempferol, and quercetin—have been isolated from *I. balsamina*. Many of these compounds or their derivatives are highly fungi-

toxic, but they are too phytotoxic to exist free in the plants. It is likely that these compounds are present in plants as glycosides or in other bound forms. The fungus apparently frees or changes the polyphenols. These are toxic to both the germ tube and the penetrated cells, thus stopping further invasion.

The ratoon stunting disease of sugarcane in Louisiana and its control with hot air. STEIB, R. J., I. L. FORBES, AND S. J. P. CHILTON. Ratoon stunting disease, first reported as occurring in Louisiana in 1951, is causing serious losses in all sugarcane-growing areas. The disease is caused by a virus that is easily transmitted mechanically. Old varieties are 100% infected, and the recently released ones are rapidly becoming infected. Highly significant losses in tonnage per acre occurred in both the plant cane and stubble crops in yield trials with old and new varieties. Losses were greater in the stubble crop than in the cane crop, especially during a year of abnormally low rainfall. Control of the disease with hot air is now being used on a commercial scale in Louisiana. After treatment according to the recommended procedure, infected "seed" material produced shoots free of the disease. In other cases where the disease was present in treated material, the incidence of infection was higher in the progeny "seed" than in the crop grown from the treated "seed". Therefore, treated "seed" is being treated again in an effort to eliminate the disease. Results have shown that after the disease is eliminated from a variety it regains some if not all of its former yielding capacity. These results support the belief that the gradual decline in yield of sugarcane varieties that has occurred throughout the world may be due to the ratoon stunting disease.

Ecology and pathogenicity studies with two widely distributed types of Fusarium oxysporum f. cubense. STOVER, R. H. The banana wilt Fusarium occurs as 2 cultural and physiological types, designated yellow and non-yellow. The former frequently is associated with pronounced yellowing of erect leaves of mature plants and the latter with leaf collapse accompanied by little or no yellowing. In culture, the 2 types differed in color, hyphal growth, sporulation, odor, and toxicity of mycelial extracts to tomato cuttings. Only the yellow type was highly virulent, causing 80% disease versus 10% for non-yellow cultures. Infection of field plants following artificial inoculation depended on high concentrations of spores in contact with roots. Root invasion in nature was invariably through secondary and tertiary roots; invasion at or near the root cap resulted in tissue discoloration in advance of intracellular colonization. Usually the fungus did not advance into the main root. Plants inoculated with single-spore yellow-type cultures frequently yielded non-yellow cultures and *vice versa;* a mixture of the 2 was occasionally found in naturally diseased pseudostems. The non-yellow type predominated in pseudostems in young plantations and the yellow type in some areas of older plantations; 80% of isolates obtained from secondary roots of all diseased and some healthy plants were the yellow type.

Factors increasing boll rots and free fatty acids in cotton seed. STREETS, R. B., A. M. BOYLE, AND H. SIMONSEN. Short periods of exposure to high temperatures and humidities in storage are known to cause marked increase in free fatty acids (FFA) in cotton seed. High FFA content occurred in many lots of Arizona cottonseed at harvest in 1954, in a smaller number in 1955, and in relatively few lots in 1956. Data from 460 samples from the 1955 crop showed a direct relation between FFA and percentage of seeds with discolored kernels. About 4% of discolored kernels gave an increase of 1% FFA. Discoloration of the kernels could usually be predicted from the condition of the short lint on the seed coat. The fungi most frequently isolated from discolored kernels were Diplodia, Aspergillus, Alternaria, and Fusarium, in that order. Infected seed came from diseased bolls. Boll rots were prevalent and severe in early pickings in some districts, scarce in the main-pickings, and increased sharply in late (especially cleanup) pickings. Boll rots become prevalent under conditions of high humidity around the cotton bolls. Remedies found effective are 1) control of excess rankness by reduced irrigation and fertilization, 2) spacing not less than 6 in. in the row in fields producing large plants, 3) bottom defoliation to improve aeration, and 4) skip-row planting (plant 4, skip 4), which greatly improves aeration as half the rows are outside rows.

The inhibition of virus infection by malachite green. TAKAHASHI, WILLIAM N. Detached Turkish tobacco leaf tissue inoculated with tobacco mosaic virus was floated inoculated side down on solutions of 4 ppm of malachite green. After exposures of 2, 4, 6, 8, 10, and 12 hours and 1 and 3 days, samples were transferred to distilled water for the duration of 6-day experiments. Control tissue was kept on distilled water and malachite green for the entire duration. At the end of the experiment, the tissue was homogenized with 0.1M phosphate buffer of pH 7.0 in the proportion of 1 g tissue to 10 ml of buffer. After appropriate dilutions were made, each sample was inoculated on half leaves of *Nicotiana tabacum* var. Xanthi,, nc., opposite the distilled-water control. Local-lesion counts showed that brief 2-4-hour malachite green treatments were as effective in inhibiting virus increase as was the 6-day malachite green control. In another series of experiments of similar design, the virus was allowed to establish infection and to increase in the tissue by the floating of inoculated tissue on distilled water before they were transferred to malachite green solution. There were indications that established infections were checked by malachite green; however, virus that was formed before the malachite green treatment was not inactivated.

A soil-column apparatus for the study of soil fungicides. THOMAS, W. D., JR. An apparatus for testing the efficacy of soil fungicides consists of a glass tube, 2.8 cm inside diameter and 10 cm long, to one end of which are attached 3 glass hooks 0.5 cm long. The inner bore has 3 0.5-cm protuberances at the bottom end upon which rests a 15-mesh wire screen disc. Attached to this tube is a glass cup 2.3 cm deep with hooks and protuberances similar to those noted above. Fine wire clamps may be used to join the 2 units. Additional length of tube can be attached with plastic tape if greater tube depth is desired. Soil is added to the tube to any depth required. Small filter-paper discs impregnated with a spore-mycelial suspension of the test fungus may be placed at any position in the soil column and in the cup. Fungicides may be mixed with the soil and leached into the cup with water. The infested paper discs may be transferred to agar to determine the fungitoxicity of the fungicide at each site of disc placement.

Hybridization between radiation-induced mutants of Diaporthe phaseolorum var. caulivora and D. phaseolorum var. sojae. THREINEN, J. T., T. KOMMEDAHL, AND R. J. KLUG. Attempts were made to cross *Diaporthe phaseolorum* (Cke. & Ell.) var. *caulivora* (Athow & Caldwell) with *D. phaseolorum* var. *sojae* (Lehman) Wehm. by use of mutants produced by irradiation with polonium 210, an emitter of alpha particles. *D. phaseolorum* var. *caulivora* is homothallic and causes stem canker of soybeans; *D. phaseolorum* var. *sojae* is heterothallic and causes pod and stem blight of soybeans. Mutants obtained by irradiating *D. phaseolorum* var. *caulivora* differed in fertility: some produced fewer perithecia than did parental types, others produced vestigial perithecia and no ascospores, and still others produced either sclerotia only or mycelia only. Perithecia were not produced in infertile mutants cultured on 14 different carbon or 27 nitrogen sources or on substrates amended with 6 vitamins; however, the number of complete or perfect

perithecia increased when relatively infertile mutants were spermatized with conidia from *D. phaseolorum* var. *sojae*. A color mutant (marker) of *D. phaseolorum* var. *caulivora* was spermatized with *D. phaseolorum* var. *sojae* and subsequent analysis of progeny indicated a cross; segregation after 2 successive generations resulted in recovery of the maternal parent (*D. phaseolorum* var. *caulivora*) but not of the heterothallic parent (*D. phaseolorum* var. *sojae*).

Strains of the cereal yellow-dwarf virus differentiated by means of the apple-grain and the English grain aphids. TOKO, H. V., AND G. W. BRUEHL. Two vector-specific isolates of the cereal yellow-dwarf virus were detected from 34 field collections. Thirty-two isolates were transmitted by both the apple-grain aphid (*Rhopalosiphum fitchii* Sand.) and the English grain aphid (*Macrosiphum granarium* Kirby). One isolate (AG strain) from volunteer barley was transmitted by the apple-grain aphid only and 1 isolate (EG strain) from wild oats by the English grain aphid only. Both vector-specific strains produced typical symptoms of cereal yellow dwarf on oats, wheat, and barley; these included mottling, discoloration, and leaf serrations. Cross-protection tests failed to show interference between the 2 virus entities, even when symptoms induced by the first strain were visible before the second was introduced. When both strains were inoculated simultaneously into young oats, no alteration in symptoms over those produced by the more virulent AG strain alone was observed. The AG strain differs from the original Washington strain of cereal yellow-dwarf virus in being more virulent on Club Mariout barley than on White Winter barley. Both the AG and EG strains were more virulent on oats and barley than on wheat.

Relation of inoculum potential of Verticillium albo-atrum to development and severity of wilt in potatoes. TOLMSOFF, W. J., AND R. A. YOUNG. Treatment of Verticillium-infested soil with Vapam (sodium *N*-methyldithiocarbamate dihydrate) resulted in disease control as measured by yield and severity of foliar symptoms. The incidence of infection was not affected, however, for *V. albo-atrum* could be isolated from stems of all plants in treated and untreated plots, even though some plants did not display foliar symptoms. To determine whether the differences in disease severity could be attributed to reduction of inoculum potential by Vapam treatment, greenhouse trials were conducted with 15 levels of inoculum ranging from 12 to 204,000 microsclerotia per cc of soil. The inoculum, grown on sand-cornmeal mixture, was added to soil previously sterilized then allowed to become reinvaded by air contaminants prior to infestation with Verticillium. Fifteen No. 10 cans were used for each inoculum level. Two suberized single-eye Russet Burbank seed-pieces were planted in each can. Isolations revealed that root infection did not occur until after initial tuber formation. Infection occurred at approximately the same time for all inoculum rates. Foliar symptoms developed and plants died more quickly at high inoculum levels than at low levels. Severity of symptoms, time of plant death, and tuber yield were related to amount of inoculum.

Biological activity of Mylone breakdown products. TORGESON, D. C., D. M. YODER, AND J. B. JOHNSON. In moist warm soil, Mylone (3,5-dimethyltetrahydro-1,3,5,2H-thiadiazine-2-thione) hydrolyzes soon after it has been applied. It was believed that the hydrolysis products or a combination of them were responsible for the fungicidal, nematocidal, and herbicidal properties of the compound. Among the materials identified were methylisothiocyanate, formaldehyde, hydrogen sulfide, and monomethylamine. These products, being chemically reactive, probably degrade further and/or react with each other. None of these materials when used alone at concentrations that might have evolved from the complete decomposition of Mylone, was sufficiently fungicidal to inhibit *Pythium* sp. in soil. Either a combined fungicidal effect or the recombination of these materials into an active fungicide is indicated.

Effect of moisture in partial-sterilization procedures. TYNER, L. E. Water in amounts of 0, 9, 10, 20, or 30% by weight was added to samples of barley seed in 1-liter flasks. Propylene oxide was added at rates of 0, 0.5, 0.75, 1.0, and 1.25 ml/liter, and the flasks were sealed for periods of 24 to 96 hours. Bacteria were not completely eliminated by any treatment, and fungi were destroyed only in the seed to which water had been added. Samples of seed moistened as above in flasks were placed in a water bath for 24 hours at 40°, 45°, 50°, 55°, or 60°C. All plated seed produced colonies of bacteria, but certain species were suppressed at 55° and 60°. Reduction in the number of colonies of fungi varied directly with the amount of water added to the seed and with the temperature applied. Species of *Mucorales* were the most persistent fungi and were susceptible to control by either propylene oxide or heat only when the treated material was moistened.

Recombination of spore color and pathogenicity between uredial clones of Puccinia recondita f. sp. tritici. VAKILI, NADER G., AND RALPH M. CALDWELL. Two uredial clones (from aecial infections of race 104B) that differed in color and pathogenicity were studied. A mixture of the red culture (race 2) and the yellow culture (race 122) was dusted heavily on the varieties Malakof, Carina, Brevit, Webster, and Loros, all of which are immune (0 type reaction) to the red and susceptible (4 type reaction) to the yellow culture. One hundred fifty-six red pustules developed from the combined inoculum on the 5 varieties immune to the red parental culture. There were an estimated 3500 parental yellow pustules and 10,000 parental red flecks to each nonparental red pustule observed. Seven of the 156 pustules were part red and part yellow. The parental red culture was free of contamination of virulent red races on the 5 resistant varieties in 2 of 3 experiments. In the third experiment, a minute trace of virulent red races (1 pustule to an estimated 250,000 flecks) was detected. The yellow culture was free of contamination by virulent red races. Totals of 16 described and 17 undescribed races were distinguished among the 67 identified nonparental clones from nonparental-type red pustules. This diversity of races was much greater than would be expected if only recombination of intact nuclei between the 2 parental races were operative as the mechanism of variation.

Elevation effects on temperature and rainfall correlated with blister rust distribution in southwestern Wisconsin. VAN ARSDEL, E. P., J. R. PARMETER, JR., AND A. J. RIKER. Blister rust was found to be ordinarily uncommon in southwestern Wisconsin, even where Ribes plants were abundant. Rust was associated with elevation as follows: below 900 ft.—practically absent; 900–1000 ft.—confined to microclimatically cool sites; 1000–1100 ft.—always present on microclimatically cool sites and occasionally elsewhere; 1100–1200 ft.—on most sites; above 1200 ft.—on all sites and occasionally severe. August daily maximum temperatures (important to teliospore fertility) decreased ¾°F for each 100-ft. increase in elevation. Above 1000 ft., the average August high was less than 81° on open field sites; at 1250 ft., it was less than 79°F. This distribution of rust in relation to temperature matched that in the state as a whole, where rust had a relatively general distribution on the cool side of a line approximating the 81° isotherm. Greater severity occurred where the August daily maximum averaged less than 79°. Rain gauges along transects in southwestern Wisconsin showed considerable increases in rainfall with increased elevation. Lower daytime temperatures and greater available moisture helped explain the relatively widespread distribution of rust on pines at higher elevations.

Effects of tomato seedlings on larvae of Meloidogyne

hapla. VIGLIERCHIO, D. R., AND B. F. LOWNSBERY. An increase in the rate of larval emergence takes place from egg masses of *M. hapla* exposed to excretions of radicles of germinating seeds of Rutgers tomato in sand or water. This hatching stimulus is much less pronounced than that induced by the hosts of some of the species of *Heterodera*, but it is statistically significant and evident within 24 hours. It is likely that the hatching stimulation provides a partial explanation for the 24-hour accumulation, noted by Wieser (1955) and the authors, of larvae of *M. hapla* on the seedling side of a predetermined diameter through the loci of egg masses in a Syracuse dish. The apparent directional movement of the larvae may well be a reaction to a chemotactic agent emanating from the roots of tomato seedlings; however, the hypothesis as yet would appear somewhat tenuous.

Results of some soil treatments for elimination of peach yellow bud mosaic virus from soil. WAGNON, H. KEITH, AND JACK A. TRAYLOR. In May, 1956, soil containing small roots was collected from 2 depths on 5 California properties from the root zone of 15 peach trees affected by yellow bud mosaic (YBM). Two composite soil samples, 1 from the top 4 in. and 1 from the next 14 in., were prepared, in a cement mixer, from the entire collection. Ten 1-gal. cans of soil from each layer were left untreated. Sixty cans of the lower soil were divided into 6 equal lots. Each of the 6 lots received one of the following treatments: 1) Nemagon (97% 1,2-dibromo-3-chloropropane), 0.5 ml by injection; 2) carbon bisulfide, 50 ml (in a sealed container); 3) Vapam (sodium *N*-methyldithiocarbamate), 10 ml mixed with the soil in a sealed container; 4) methyl bromide, 5 lb. per 1000 cu. ft. of container space for 24 hours; 5) D-D (1,3-dichloropropane; 1,2-dichloropropane), 1 ml, and 6) steam, 20 lb./sq. in. for 1 hour. Each of the 80 cans was planted with 8 Lovell peach seeds. In April, 1957, YBM showed in 5 of 10 cans and in 25 of 57 seedlings that grew in the untreated lower soil; no YBM showed in the 425 seedlings that grew in the treated soil or in the 53 seedlings that grew in the untreated upper soil.

Studies of internal tipburn of cabbage. WALKER, J. C., AND L. V. EDGINGTON. Internal tipburn of cabbage is a necrotic breakdown beginning in the marginal tissue of leaves located near the center of the head. The disease appears as the plants are approaching maturity. It is most severe in plants located in poorly drained portions of fields. No disease occurred in plants grown to head stage in sand watered with balanced Hoagland solution containing 200 ppm calcium. The disease was severe in comparable plants supplied with the same solution except that the calcium content was 25 ppm. Analysis of cabbage heads showed that the percentage of calcium in the marginal tissue of internal head leaves was about one-third the average for the total head tissue. This difference was observed in diseased and in healthy heads. It is suggested that the breakdown in the low-calcium tissue is associated with environal factors that upset the normal flow of slow-moving calcium ions into the marginal leaf tissue.

Septoria leafspot of marigold, Tagetes erecta. WEBER, GEORGE F., AND WINIT CHANGSRI. During the summer and fall of 1956, several garden plantings of tall-growing varieties of marigold in Gainesville, Florida, were severely diseased by a leaf-spotting fungus, *Septoria* sp. The disease first appeared on the lower leaves of blossoming plants that were about 3 ft. tall. As the season progressed the disease became more severe and within 6 weeks from first appearance the foliage of the plants was killed. Lesions were noticeable when less than ¼ mm in diameter as slightly sunken spots (light tan to brown) scattered over both surfaces of the blade. By the time the lesions were more than 1 mm in diameter, host cells had collapsed and the surfaces had become shiny and brown. Immediately after host cells were killed, pycnidia of the fungus appeared as minute black specks. As the lesions enlarged and dried, the contour of the leaflets became distorted. Entire leaflets frequently were involved in single lesions up to 2–3 mm long. The pycnidia, 60–80 μ in diameter and with small ostioles, are black, circular, slightly flattened, substomatal and usually most plentiful on the lower surface. The pycnidiospores are long, slender, slightly bent or curved, cylindrical, and hyaline. They usually are 3 septate and measure 20–30 $\mu \times$ 1–2 μ.

Temperature influence on the rate of passage of cucumber mosaic virus through the epidermis of cowpea leaves. WELKIE, GEORGE W., AND GLENN S. POUND. Primary leaves of cowpea were inoculated, detached, and incubated on water agar in closed containers at 16°, 20°, 24°, and 28°C. At hourly intervals, a portion of the epidermis was removed from the left half of each of several leaves. The number of lesions produced in unit areas devoid of epidermis was compared with that in equal areas on the opposite leaf-half. At any 1 temperature, lesion numbers increased in the area devoid of epidermis as the time between inoculation and removal of epidermis was extended. Progressively more incubation time, prior to removal of epidermis, was required for any lesions to develop in the stripped areas as the incubation temperature was decreased. Also, progressively more time was required for equal numbers of lesions to develop in stripped and unstripped areas as the incubation temperature was decreased. Results suggest that temperature directly influences rate of virus passage through epidermal cells into mesophyll cells. Optimum temperature for maximum lesion production was lower (20°) than that which induced the most rapid rate of virus passage through the epidermis (28°). Results of experiments with whole plants indicated that low internal temperatures of intact leaves diminished the influence of high external air temperatures on rate of virus passage through epidermis.

Anastomosis of germtubes in Puccinia graminis. WILCOXSON, ROY D., J. F. TUITE, AND SHIRLEY TUCKER. Germtube fusion among biotypes, races, and varieties of *Puccinia graminis* is common, but environmental factors affect the frequency and nature of anastomosis. Fusion was rare and no fusion bodies formed when spores germinated in mineral oil, whereas both were common on 6 different media. Numerous germtube fusions and occasional appressorial fusions and fusion bodies formed on wheat seedlings when spores in water were applied to leaf surfaces. On wheat leaves and on nutrient agar, the fusions frequently formed a network. Osmotic concentrations of the supporting medium at 5–7.5 atmospheres appeared to favor fusion-body production. By use of yellow urediospores as markers and of a phase microscope, fusions were observed to occur within biotypes, between races, and between the variety *tritici* and the varieties *avenae*, *agrostidis*, *poae*, and *secalis*. The germtubes contained 2–11 nuclei; these nuclei were not associated in pairs and were usually concentrated in the tip of the germtube. Nuclei appeared to divide singly and not in pairs as is normal in older mycelium. In fusion bodies, 2 and 4 nuclei were commonly seen along with protoplasmic materials that apparently were moving between germtubes and fusion bodies.

Methyl bromide gives promising control of broomrape. WILHELM, STEPHEN, LEE C. BENSON, AND JAMES E. SAGEN. *Orobanche ramosa* L., the clandestine root parasite of many herbaceous plants, causes serious losses in lettuce and tomato in the Alvarado area of California. Attempts to kill its minute seed in soil have been successful only by fumigation with methyl bromide. Applied by the MacLean Blower under polyethylene tarpaulins to moist listed soil at rates above ½ lb. per 100 sq. ft., methyl bromide has generally reduced the infestation of broomrape in ¼-acre plots to fewer plants than occur in 1 sq. ft. of nonfumigated soil, i.e. to fewer than 100 plants. Commercial Weedfume, a solution containing 70% methyl bromide,

gave excellent control when chiseled in at 250 lb. actual CH_3Br per acre followed by covering with tarpaulins but not when followed by rolling or by water sealing. No control was obtained with chloropicrin, CS_2, or Dithane D-14 (19% nabam) at rates up to 45, 800, and 120 gal. per acre, respectively; with calcium cyanamide, Mylone (85% 3,5-dimethyltetrahydro-1,3-5,2H-thiadiazine-2-thione), Crag DCU 73W (73% dichloral urea), or Vapam (sodium *N*-methyldithiocarbamate dihydrate) at rates to 1000, 600, 35, 600 lb. per acre, respectively; with manure up to 60 tons per acre; or with iron sulfate sufficient to lower the pH to below 5. Plowing that was deep, but inadequate to bury all of the seed below 12 in., reduced the infestation and may offer promise of control in light soils of uniform vertical texture.

Acquisition and test feeding periods required on Physalis floridana and Datura stramonium for aphid transmission of potato leafroll virus. WILLIAMS, W. LLEWELYN, AND A. FRANK ROSS. The minimum time required for acquisition of leafroll virus by *Myzus persicae* from infected *Physalis floridana* or *Datura stramonium* was ½ hour. Groups of 5 aphids given such acquisition periods did not transmit the virus to seedlings of *D. stramonium* during test feedings of ½ hour or more, but some groups transmitted to seedlings of *P. floridana* during the first ½ hour of test feeding. Aphids reared on infected *D. stramonium* effected transmission to *D. stramonium* and *P. floridana* in 10 and 3 minutes, respectively. Such aphids transmitted the virus with equal facility to the 2 species during test feedings of 15–30 minutes. The total time necessary for transmission from *D. stramonium* to *D. stramonium* decreased as the duration of acquisition feeding was increased; the shortest total transmission time was 17–29 hours, consisting of acquisition and test feeding periods of 17 and 12 hours, respectively. Following acquisition feedings of 1, 5, and 10 hours, aphids did not transmit during the first 24, 36, and 12 hours, respectively, of test feeding on *D. stramonium*. The explanation for failure to obtain transmission to *D. stramonium* when short acquisition and test feeding periods were used appears to be that this species is relatively resistant to small doses of the virus.

Aphid transmission of potato leafroll virus as affected by the feeding of nonviruliferous aphids on the test plants and by vector variability. WILLIAMS, W. LLEWELYN, AND A. FRANK ROSS. The previous report that the probability that a single aphid (*Myzus persicae*) can effect transmission of leafroll virus decreases as the number of aphids per plant is increased was confirmed. The depression in individual efficiency occurred with both 24-hour and 5-day test feedings on *Physalis angulata*; it appears to result in part from interference by a colony on the feeding of individuals and in part from an effect of aphid feeding on susceptibility of the test plant. Susceptibility of the seedlings was not greatly affected by the feeding of nonviruliferous aphids (5–10 per plant) for 5 days before or after an inoculation feeding of 15 hours or 5 days, but it was lessened appreciably by the feeding of 5 or more nonviruliferous aphids for 12 days prior to an inoculation feeding of 5 hours. These data suggest that the susceptibility of *P. angulata* is decreased by aphid feeding but that this effect is not great enough to explain entirely the relative inefficiency of individuals in a colony. The marked variation in ability of individuals to transmit leafroll virus appears to be due to heritable characters. The progeny of individuals that were good transmitters were efficient vectors, and those of poor transmitters were inefficient vectors.

Initial and subsequent control of radish yellows by various treatments during eight successive plantings. WILSON, J. D. Several chemical compounds, such as methyl bromide, formaldehyde, and ethylene dibromide, which gave comparatively good control of radish yellows (caused by *Fusarium oxysporum* f. *conglutinans*) in the first planting following soil treatment, were not effective in later plantings. Terraclor (75% pentachloronitrobenzene) and PRD (3,4-dichlorotetrahydrothiophene 1,1-dioxide) encouraged the development of the disease in both rapidity and percentage above that which occurred in the untreated soil. Treatments that gave fair control through the fourth planting (about 70–80 days after soil treatment) but were ineffective in the seventh and eighth included mixtures of Mylone (3,5-dimethyltetrahydro-1,3,5,2H-thiadiazine-2-thione) plus Vapam (sodium methyldithiocarbamate) and EDB (ethylene dibromide) plus Picfume (trichloronitromethane), all used at only ½ the concentrations of single rates. Similar mixtures of Mylone and Picfume and of Vapam and Picfume continued to be quite effective in the last 2 plantings. Only 3 of the 20 compounds used in 40 different treatments were still giving good control in the last (eighth) planting, made about 130 days after treatment. Of these, chloropicrin was the most durable and effective, followed in turn by Mylone and Vapam. Even a standard treatment with steam at a temperature of 180°–200°F for 2 hours failed to give worthwhile control of this disease beyond the fourth planting, because of a gradual build-up of the causal organism.

Bean halo blight control with streptomycin sulphate. WOOLLIAMS, G. E. Field-sown bean plants grown in 1955 from seed infected with the halo blight pathogen (*Pseudomonas phaseolicola*) received 3 weekly spray applications of streptomycin sulphate (500 ppm). The first spray was applied after the first pair of trifoliate leaves had developed and after halo blight symptoms had appeared on the foliage. Diseased plants were rogued from all plots just before the second spray application. By the time the seed crop was harvested in the fall, halo blight had reappeared on at least 25% of the unsprayed check plants but on none of the sprayed plants. In replicated plots planted in 1956 with this seed, 36% of the plants grown from seed that had been collected from the unsprayed check plots had halo blight; only 7% of those grown from seed that had been collected from the sprayed plots had the disease. In a replicated seed-treatment experiment, diseased bean seed was soaked for 30 minutes, just prior to field planting, in streptomycin sulphate solutions at concentrations of 50, 100, 250, and 500 ppm. Average halo blight incidence was 56, 49, 36, and 38%, respectively among the plants grown from treated seed; 72% of the plants grown from untreated seed were infected.

Variation in Sclerotinia sclerotiorum induced by irradiation. WYLLIE, T. D., AND J. M. WALTER. Ascospores, mycelial fragments, and sclerotia of *Sclerotinia sclerotiorum*, when irradiated by alpha particles and by ultraviolet light, yielded many distinct variants. Variants differed from the parental stock in color, in rate and type of growth, and in size, shape, number, and location of sclerotia. A few variants produced no sclerotia on any of the substrates. Some produced approximately twice as many apothecia per sclerotium as did the parents, whereas others produced fewer apothecia than did the parents. Preliminary tests indicated marked differences in pathogenicity. Injurious effect of irradiation may persist for some time in the fungus: some of the transplants grew very little, others survived for 1 or more successive transfers before dying, and still others recovered completely, although retarded initially.

Heat tolerance of virus-infected and fungus-infested tissues. YARWOOD, C. E. When bean leaves infected with tobacco mosaic virus (TMV), tobacco ring spot virus, or peach yellow bud mosaic virus were heated about dawn for 25–40 seconds at 50°C in water at 2–3 days after inoculation, only the noninfected tissue was severely injured. When the noninfected tissue had lost its green color (about 2 days after heating), the infected areas appeared as green islands or as green halos surrounding necrotic centers. When similar leaves were heated in the afternoon, no such green islands appeared. When TMV-infected leaves were similarly heated more than 3 days after inoculation, necrotic halos frequently appeared around the virus lesions

and about 1 mm beyond the margins of the lesions. When bean leaves lightly infected with the uredinial stage of *Uromyces phaseoli* were heated at dawn 3–8 days after inoculation, the rust fungus and the healthy tissues were killed, but the pustule areas and the areas beyond the mycelium remained as green islands in the necrotic tissue. These results are interpreted in terms of heat protection as a result of abnormally high accumulation of carbohydrate around the lesions.

Contact transmission of peach ring spot virus. YARWOOD, C. E. A strain of peach ring spot virus from apricot in Solano County, California has been mechanically transmitted to Pinto and to certain other bean varieties. When 1 of 2 bean plants in a pot was inoculated, the noninoculated plant in the same pot frequently became infected. No transmission occurred when the foliage of 2 such plants, with roots intermingled, was separated by a glass barrier before inoculation. When healthy bean foliage was rubbed with foliage of infected bean plants in separate pots, transmission of the virus occurred in 21 of 76 inoculations, though infection also occurred in 4 of 43 noninoculated controls. When foliage of healthy peach seedlings, was rubbed with foliage of infected beans or infected peaches, 2 of 37 peach seedlings became infected. There was no infection in 29 controls. Other strains of peach ring spot virus have not been transmitted by contact.

Comparative protection of bean leaves from fungus infection by antibiotic treatments of lower and upper surfaces. ZAUMEYER, W. J. Treating lower surfaces of bean leaves with either oligomycin or anisomycin protected them better against infection by *Uromyces phaseoli* var. *typica* than did treating upper surfaces. Leaves painted with oligomycin at 100 and 50 ppm on their lower surfaces and inoculated with spores 24 hours later on the upper surfaces averaged 0.6 and 7.1 pustules per leaf, respectively, compared with 15 and 150 pustules respectively, on leaves treated on upper surfaces and inoculated on lower. Inoculation of lower and upper surfaces of untreated leaves produced 306 and 250 pustules per leaf, respectively. Leaves treated with anisomycin at 100 and 50 ppm averaged 3.5 and 23 pustules per leaf, respectively, when treated on lower surfaces and 133 and 206, respectively, when treated on upper surfaces. Untreated leaves inoculated on lower and upper surfaces averaged 505 and 317 pustules, respectively. Excellent and good protection against infection by *Colletotrichum lindemuthianum* resulted from treatment of lower surfaces with 100 and 50 ppm of oligomycin respectively. Upper-surface treatment gave no protection. Untreated leaves became seriously infected regardless of the surface inoculated.

Phytoactin and phytostreptin, two new antibiotics for plant disease control. ZIFFER, JACK, S. J. ISHIHARA, T. J. CAIRNEY, AND A. W. CHOW. Two polypeptide antibiotics phytoactin and phytostreptin, were isolated from fermentation whole cultures of an undescribed species of *Streptomyces* and its variant, respectively. Both antibiotics are active against fungi and gram-positive bacteria. In greenhouse tests, low concentrations of both antibiotics have given effective control, without host injury, of tomato early blight, tomato late blight, and bean rust. Both are soluble in methanol, ethanol, 1-butanol, chloroform, acetone, methylisobutyl ketone, and other organic solvents. Both are slightly soluble in ether and insoluble in petroleum ether. Phytoactin is slightly soluble in water, and phytostreptin is completely soluble in water. Paper chromatographic analyses of acid hydrolyzates indicated that both contain valine, a-alanine, proline, leucine (or isoleucine), arginine, glycine, and serine. Electrometric titration revealed the presence of an apparent free carboxyl group in both antibiotics and an apparent free amino group in phytostreptin. Both antibiotics are heat stable and are not digested by pepsin, trypsin, or purified bacterial or fungal proteases. Phytoactin dialyzes through cellophane into aqueous methanol, phytostreptin into water. Salts and complexes of phytostreptin were active *in vitro* and in greenhouse tests.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief description of the contents of the books will be included.

FISCHER, G. W., AND C. S. HOLTON. 1957. **Biology and control of the smut fungi.** The Ronald Press Company, New York. 622 p. Price $10.00.

The knowledge that man has gained of the smut fungi and of their impact on human welfare is presented, in condensed form, in this comprehensive volume. In addition to previously published information, the authors include many items of their own that have not been published previously. The book begins with a chapter on morphology, taxonomy, and symptomatology; this includes a host family register of more than 1100 species in 33 genera, with species catalogued by host family. Subsequent chapters deal with history and economic importance, nomenclature and phylogeny, secondary effects on the host, variations of the smut fungi and varietal reactions of the hosts, direct effects of smut fungi on man and animals, methods and techniques used in the study of smut fungi, and control practices. An extensive bibliography, occupying 147 pages, is included.

WOLF, F. A. 1957. **Tobacco diseases and decays.** Duke University Press, Durham, North Carolina. Ed. 2, revised. 396 p. Price $7.50.

This is a revision and enlargement of the edition published in 1935. The author has assembled information from all parts of the world on matters relative to the diseases of cultivated tobacco and to decays of tobacco after harvest. The introductory chapter provides general information on tobacco and on plant diseases. Several chapters are devoted to subjects dealing with seedbed problems and management, chemical injuries, weather as a factor in disease, nutritional diseases, and abnormalities due to inherited factors. Later chapters provide detailed information on diseases of the growing crop caused by viruses, bacteria, fungi, nematodes, and flowering plants. The final chapter deals with decays during curing, during aging and fermentation, during storage, and after processing.

HELMINTHOSPORIUM NODULOSUM AND RELATED SPECIES[1]

E. S. Luttrell[2]

SUMMARY

Helminthosporium nodulosum Berk. & Curt. ex Sacc. has been misinterpreted, the name having been commonly applied to an unrelated fungus similar to *H. sacchari* Butl. Study of the type collection and of authentic fresh material has shown that *H. nodulosum* is characterized by straight, obclavate, brown, thin-walled, 3- to 7-septate conidia and conidiophores that are swollen at the tips and at the nodes and tend to remain hyaline until fully developed. It causes seedling blight, leaf stripe, and sooty heads of *Eleusine spp.* Infection is systemic, although it may occur through immature tissues at any stage in the development of the host until it approaches maturity. *H. leucostylum* Drechs. is a synonym.

Helminthosporium kusanoi Nisikado and *H. hadrotrichoides* Ell. & Ev. are distinguished from *H. nodulosum* by their parasitism on *Eragrostis* spp. and their shorter, fewer-septate conidia. Conidia of *H. kusanoi* are 1- to 6-septate. In *H. hadrotrichoides*, the conidia are consistently 3-septate, and the conidiophore tips are more prominently swollen than in *H. kusanoi.*

The perithecial stage of *H. nodulosum* was produced in culture by mating compatible strains of the fungus. Isolates from the field were approximately equally divided between 2 sex-reaction groups. The perfect stage is described as a new species, *Cochliobolus nodulosus.* It is most closely related to *C. kusanoi* (Nisikado) Drechs. ex Dast.

From a review of the literature, it is obvious that there has been confusion in the application of the name *Helminthosporium nodulosum* Berk. & Curt. ex Sacc. Entirely different fungi have been referred to this species by Nisikado (15), by Butler (3), and by Mitra and Mehta (13). Other species, including *H. leucostylum* Drechs. (7), *H. kusanoi* Nisikado (14), and *H. hadrotrichoides* Ell. & Ev. (21), appear to be closely related to *H. nodulosum* as originally illustrated by Berkeley (1). In order to determine the identity and relationships of these species, a study was made of the morphology, life cycle, and parasitism of *H. nodulosum* from fresh authentic material on *Eleusine indica* (L.) Gaertn. collected in Georgia and Virginia. This material was compared with available type collections, herbarium specimens, and cultures of the species involved in this complex.

METHODS.—Studies were made of conidia and conidiophores produced on the host and in culture in Petri plates of 20 per cent V-8 juice[3] agar, of ground barley straw sterilized with propylene oxide and scattered over the surface of water agar, and of fresh host leaves and seed heads sterilized with propylene oxide on moistened filter paper. In attempts to produce the perfect stage, soaked corn grains and fragments of barley straw were arranged in Petri dishes that were placed in a sealed jar with 1 ml propylene oxide per liter capacity for 24 hours. Sachs agar (19) was poured into the dish until the gas-sterilized corn grains and barley straw were partially immersed. Corn and barley grains surface sterilized with mercuric chloride and placed in plates of Sachs agar as recommended by Shoemaker (19) and Tinline (22) were used also. Transfers to plates were made with drops of suspensions of mixtures of conidia in sterilized water or with blocks of mycelium. The plates usually were held in an incubator at 25°C for 1 week and then transferred to a 20° incubator for 3–4 weeks. Inoculations were made on potted host plants by spraying them with suspensions of conidia scraped from cultures on sterilized leaves or of conidia and mycelial fragments from shreded plate cultures. The inoculated plants were placed in an inoculation chamber provided with a humidifier at a temperature of approximately 28°C for 48 hours and then returned to the greenhouse benches.

SYMPTOMS AND MORPHOLOGY.—On *Eleusine indica*, *Helminthosporium nodulosum* caused seedling blight, spotting and striping of the leaves, and sooty heads of the inflorescence. Young seedlings were killed, shriveled, and blackened with the conidiophores and conidia of the fungus. Leaf spots first appeared as elliptical, oblong, or long rectangular areas of grayish water-soaked tissue. The lesions became straw colored and had narrow reddish-brown borders. They elongated and coalesced to form stripes extending nearly the full length of the blade. The leaves died back and became frayed at the tips (Fig. 1) In moist weather, the lesions were covered with a compact layer of short conidiophores and conidia and closely resembled stripe smut symptoms. Infected inflorescences were stunted, failed to exsert stamens, produced no viable seed, and were covered with a black woolly growth of conidiophores and conidia.

Conidia in all collections and in culture were straight, ellipsoid, ovate, or typically obclavate, thin walled, and pale to moderately dark brown or olivaceous brown (Fig. 2, 4, 5, 6). The circular hilum was included within the contour of the rounded basal wall and was

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Paper No. 312, Journal Series, Georgia Experiment Station.

[2] I am grateful to Sir Edward Salisbury, Royal Botanic Garden, Kew, to Mr. J. H. Stevenson, National Fungus Collections, Beltsville, to Dr. I. M. Lamb, Farlow Herbarium, Harvard University, to Dr. D. P. Rogers, New York Botanical Garden, and to Prof. E. Castellani, Instituto di Patologia Vegetale, Universita di Torino, for their kindness in furnishing specimens from their respective herbaria and in giving other assistance.

[3] A blend of juices from tomatoes, carrots, celery, beets, parsley, lettuce, spinach, and watercress (Campbell Soup Company).

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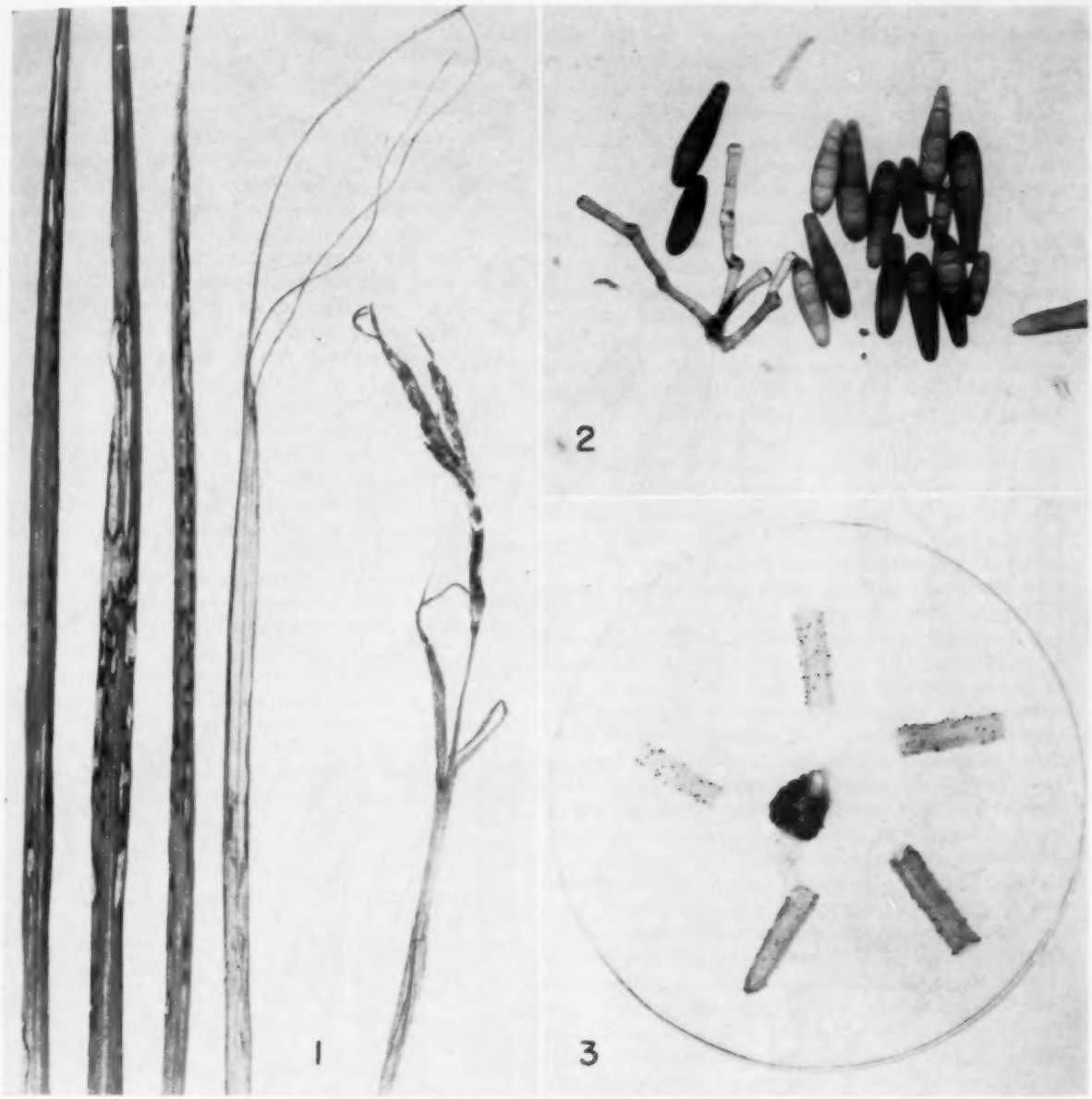
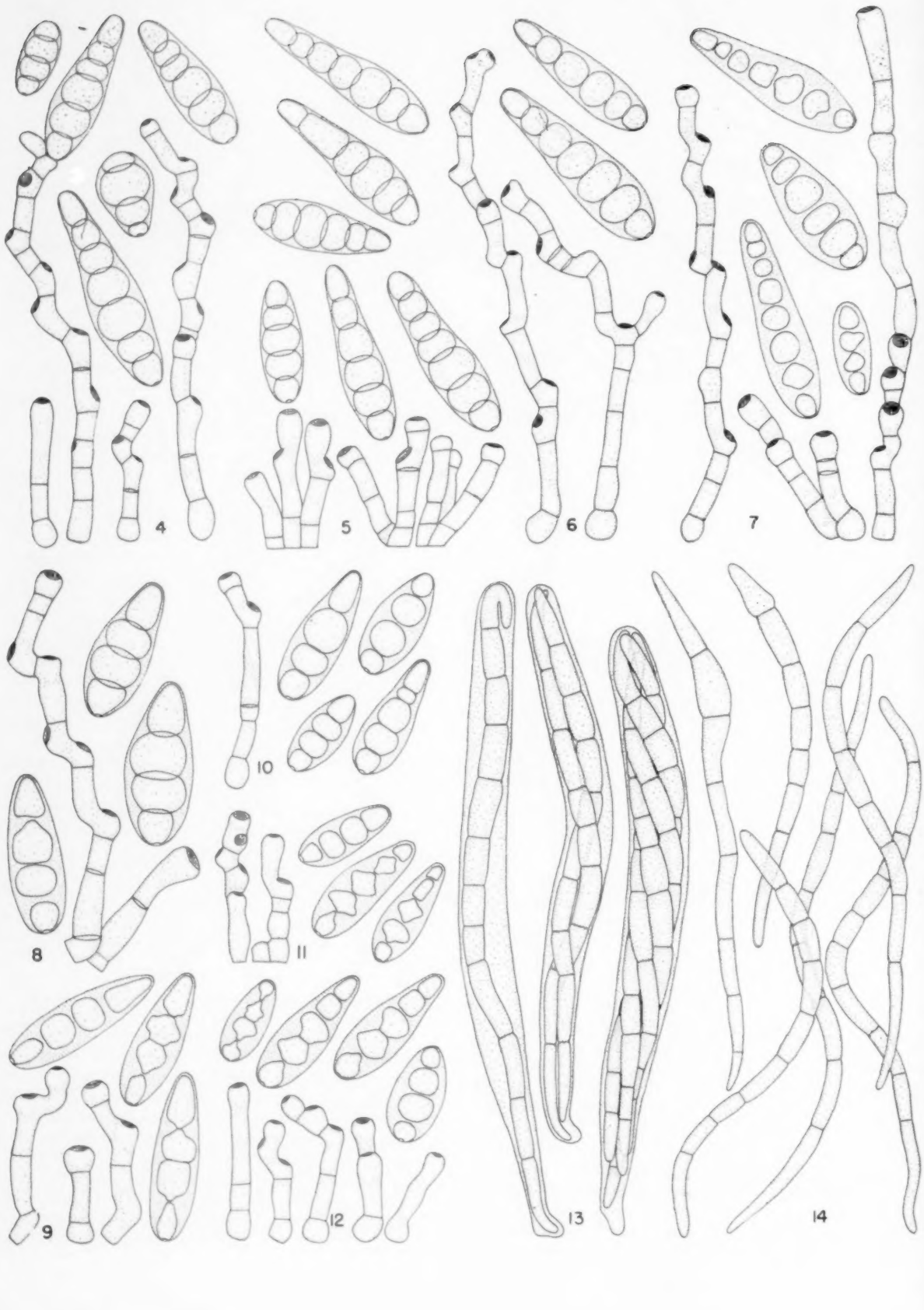


FIG. 1–3. Fig. 1. Leaves and seed head of *Eleusine indica* infected with *Helminthosporium nodulosum*.—Fig. 2. Conidia and conidiophores of *H. nodulosum* from leaf of *E. indica*.—Fig. 3. Perithecia produced in culture on gas-sterilized barley straw and corn grain by cross of isolates No. 1060-2 and 1060-3 of *H. nodulosum*.

often surrounded by a hyaline area. Conidia measured 36–70 × 10–18 μ (means 48.4–56.6 × 13.0–15.7 μ) and were 4- to 7-septate (means 5.0–5.3) with length/width ratios of 3.6–3.7. They germinated by polar germ tubes from the tip of the apical cell and just to one side of the hilum in the basal cell.

Conidiophores on leaves (Fig. 5) were simple, cylindrical, and 2- to 4-septate. They remained very short, measuring 19–42 × 4.2–5.6 μ, with swollen tips 6.2–7.8 μ in diameter. The conidiophore proliferated from the side beneath the terminal conidial scar and produced a succession of conidia. The circular conidial scars occurred at close intervals on swollen nodes that gave the conidiophore a nodulose appearance. The conidiophores at first were hyaline throughout but ultimately became fuligenous or pale brown toward the base. They arose singly or in compact clusters directly through the epidermis from swollen basal cells, often so compacted that they gave the appearance of a basal stroma.

On inflorescences, the conidiophores became much longer than those on leaves, measuring 34–143 × 5.0–5.9 μ and 5.6–7.0 μ in diameter at the tips. They were 1- to 12-septate (Fig. 4) and occasionally were branched.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Branching usually resulted from proliferation from 2 sides beneath the terminal scar, the conidial scar remaining in the crotch of the Y-shaped branch thus formed. Young conidiophores were hyaline throughout. Older conidiophores were hyaline at the tips, but the lower portions became moderately dark brown, approximating the color of the conidia. Conidiophores in culture (Fig. 6) were similar to those on the inflorescences, measuring 28–148 × 5.0–5.6 μ with swollen tips 5.6–8.4 μ in diameter. They were 1- to 15-septate. On 20 per cent V-8 juice agar, single-conidium isolates varied greatly in cultural characteristics, ranging from types with closely appressed black mycelium producing abundant conidia to nonsporulating types with fluffy white mycelium. Linear growth rates per day at 25°C varied from 1.2 to 4.5 mm.

PERFECT STAGE.—When appropriate strains of *Helminthosporium nodulosum* were crossed, perithecia typical of the genus *Cochliobolus* were produced on sterilized barley straw and on corn and barley grains but rarely on the surrounding agar (Fig. 3). They were produced most abundantly on corn grains but often were obscured by an overgrowth of mycelium. Although less abundant on barley straw than on corn grains, the perithecia were accompanied by little aerial mycelium. Perithecial production was poorest on barley grains.

Perithecia were first obtained in a cross of isolates No. 882, 965, and 992.[4] From these perithecia, 8 single-ascospore isolations were made. When these isolates were crossed in various combinations, 4 proved to be of 1 sex reaction type and were arbitrarily labelled "A" strains; 4 were of the opposite type and were labelled "a" strains. Two compatible isolates, No. 1060-2 ("A" strain) and No. 1060-3 ("a" strain) were selected for use as testor strains in determining the sex reaction of other isolates. Isolates from Plains, Ga., (No. 964) and Experiment, Ga., (No. 965, 966, 967, 968) proved to be "A" strains. Isolates from Blairsville, Ga., (No. 992), Experiment, Ga., (No. 882, 1107), Cairo, Ga., (No. 1177), Richmond, Va., (No. 976, 977), and Beltsville, Md., (No. 1003) proved to be "a" strains. One isolate (No. 975) from Richmond, Va., did not produce perithecia when crossed with either of the testor strains or with other isolates.

The superficial perithecia were black, globular, and 276–414 μ in diameter, with long cylindrical beaks 97–262 × 55–83 μ. The asci, interspersed with pseudoparaphyses, were 1- to 8-spored, cylindrical, and measured 120–193 × 14.0–16.8 μ (Fig. 13). The ascospores (Fig. 14) were vermiform, hyaline, and coiled in a helix in the ascus. On corn grains they measured 98–193 × 3.9–7 μ (mean 137.4 × 5.6 μ) and were 4- to 12-septate (mean 8.3); on barley straw they measured 90–179 × 4.2–7.8 μ (mean 144.6 × 5.4 μ) and were 3- to 11-septate (mean 6.9). Although normal ascospore discharge was not observed, in water mounts the ascus split across the apex and the ascospores oozed out through the cleft, uncoiling and separating as they emerged.

PARASITISM.—Observations in the field showed that when plants of *Eleusine indica* were infected the entire plant usually was stunted and every leaf and inflorescence was affected, whereas adjacent plants might show no trace of infection. Occasionally lop-sided plants were found in which some of the tillers were infected and stunted and others were entirely healthy. The appearance of the plants suggested the possibility of systemic infection. To explore this possibility, 6 infected plants were dug in the field and placed in pots in the greenhouse where conditions were not favorable for reinfection. All diseased top growth was cut back and removed. All new growth from these plants exhibited typical symptoms. Isolations were made from surface-sterilized tissue from the crowns, from inflorescence primordia, and from basal portions of leaves where no lesions were present. *Helminthosporium nodulosum* was consistently isolated from all parts of infected plants except the roots.

Inoculations with a mixture of conidia from Isolates No. 964 and 965 were made on 2 series of plants of *E. indica* and *E. coracana* (L.) Gaertn. grown in 6-in. pots in the greenhouse. In the first series, seedlings were inoculated as they began to appear above ground 4 days after planting; in the second, mature plants were inoculated. There were 2 replications in each series. There was no infection of controls, none on *E.*

[4] Numbers are accession numbers in the author's culture collection. All cultures were isolated by the author unless another source is indicated.

FIG. 4–14. Fig. 4–7. Conidia and conidiophores of *Helminthosporium nodulosum*.—Fig. 4. From seed head of *Eleusine indica*.—Fig. 5. From leaf of *E. indica*.—Fig. 6. From culture on gas-sterilized barley straw.—Fig. 7. From Type Collection, Herb. Berkeley 1879, Royal Botanic Garden, Kew.—Fig. 8–9. Conidia and conidiophores of *H. hadrotrichoides*.—Fig. 8. From Type Collection, Herb. A. Commons, on leaves of *Eragrostis major*, Faulkland, Del., 17 Sept., 1886, New York Bot. Gard.—Fig. 9. From leaves of *E. cilianensis*, H. C. Greene, Madison, Wisc., 11 Aug., 1947, National Fungus Collections.—Fig. 10–12. Conidia and conidiophores of *H. kusanoi*.—Fig. 10. From culture No. 1023 (Nisikado strain from Centraalbureau voor Shimmelcultures) on gas-sterilized leaf of *Eleusine indica*.—Fig. 11. From leaves of *Eragrostis* sp., R. Sprague, Ames, Iowa, 10 Sept., 1940.—Fig. 12. From leaves of *Eragrostis* sp., R. Sprague, Miles City, Mont., 13 Aug., 1941, National Fungus Collections.—Fig. 13–14. Asci and ascospores of *Cochliobolus nodulosus* produced in culture on gas-sterilized barley straw.

TABLE 1.—*Infection by Helminthosporium nodulosum of plants of Eleusine indica inoculated at various stages of growth*

Age of plants at inoculation	No. plants inoculated	Percentage of plants emerged	Percentage of plants infected and killed			
			Two weeks		Eight weeks	
			Infected	Dead	Infected	Dead
Control	200	86	0	0		
Seed	200	72	83	75	100	94
Five days	278		55	23	98	80
Ten days	179		77	16	99	73
Three weeks	105		78	0	99	18
Five weeks	96		55	0	100	0

coracana in either series, and none on mature plants of *E. indica*. Of 468 inoculated seedlings of *E. indica*, 26 per cent apparently were healthy at the end of 2 weeks, 51 per cent were infected but still living, and 23 per cent were dead. In these same pots percentages of seedlings in each category at the end of 9 weeks were as follows: healthy—1 per cent, living infected plants—4 per cent, dead—95 per cent.

To determine the period of susceptibility of *E. indica*, inoculations with a mixture of conidia from Isolates 964 and 965 were made on the following series: 1) seed placed on the surface of soil, sprayed with inoculum, and covered with soil; 2) seedlings inoculated 5 days after planting, when the first leaf was just emerging; 3) 10-day-old plants in the 2-leaf stage; 4) 3-week-old plants in the 4- to 5-leaf stage; and 5) 5-week-old plants with 7–8 leaves and beginning to form tillers. Each treatment was replicated twice. Results at the end of 2 and 8 weeks are in Table 1. Inoculation of seed caused a slight reduction in emergence. There was severe infection of plants inoculated at all stages of growth, but there was progressively less killing with increasing age of plants at the time of inoculation. Infection on plants 5 weeks old when inoculated was largely confined to the tillers.

To determine variation in virulence among isolates of *H. nodulosum*, 10-day-old seedlings of *E. indica* in the 2-leaf stage were inoculated with spore suspensions of each of 7 isolates. Results are in Table 2.

When seed or very young seedlings were inoculated with virulent strains, the majority of the plants died in the seedling stage. The leaf tips wilted, the leaf tissue became grayish and water soaked, and the whole plant died back. Others showed no symptoms initially, and the first lesions appeared on the third, fourth, or fifth leaf. In this case, typical leaf spot and stripe symptoms were produced. On occasional plants, expression of symptoms was delayed until the plant began to head. Plants exhibited varying degrees of stunting, depending on the delay in symptom expression. All inflorescences from infected plants were blighted. All tillers from plants infected in the seedling stage were infected. When older plants were inoculated, only the new growth showed symptoms. In plants approaching maturity at the time of inoculation, there was little infection of the primary culm except for the uppermost leaves, but 1 or more of the tillers were infected. Such individual infection of tillers in older plants may explain the development of the lop-sided, partially infected plants observed in the field.

TAXONOMY.—*Helminthosporium nodulosum*.—This species is based on a collection on seed heads of *Eleusine indica* by M. A. Curtis in South Carolina. It was first published as *H. nodosum* Berk. & Curt. (1) without description but with drawings of conidia and conidiophores. A brief description of *H. nodosum* appeared some years later (2). Because of a prior homonym (*H. nodosum* Crouan), Saccardo (18) proposed the new name *H. nodulosum* which he attributed to Berkeley and Curtis.

The type collection was obtained from the Herbarium of the Royal Botanic Garden at Kew. Conidia in the type collection were straight, ellipsoid, ovate, or typically obclavate, 3- to 7-septate (mean 5.2-septate), and $28–64 \times 12–17\,\mu$ (mean $48.6 \times 14.0\,\mu$), with a length/width ratio of 3.5 (Fig. 7). The conidiophores (Fig. 7) formed black woolly mats over the surface of the glumes. They were cylindrical, simple or occasionally branched, hyaline at the tips to dark brown in the lower portions, 3- to 11-septate, $34–154 + 5.0–5.6\,\mu$, $5.6–8.4\,\mu$ in diameter at the swollen tips, and swollen and geniculate at the conspicuous circular conidial scars that occurred in close succession in the upper portions.

According to Nisikado's (14) description, material he collected on *E. indica* in Formosa and referred to *H. nodulosum* was similar to that in the type collection; the name in this case was correctly applied. In other reports, however, it has been misapplied. Butler (3) placed under *H. nodulosum* a fungus on *E. coracana* in India with fusiform, straight or curved, 4- to 10-septate, brown conidia measuring $50–100 \times 12–17\,\mu$. Mitra and Mehta (13) followed Butler in referring a similar fungus causing leaf spot of *E. coracana* in

TABLE 2.—*Infection of 10-day-old seedlings of Eleusine indica following inoculation with various isolates of Helminthosporium nodulosum*

Isolate	No. plants inoculated	Percentage of plants infected and killed			
		Three weeks		Seven weeks	
		Infected	Dead	Infected	Dead
Control	175	0	0	4	0
964	154	92	37	100	76
965	123	85	69	99	89
968	197	82	55	99	83
976	149	82	11	96	58
977	175	6	0	20	15
992	152	27	2	29	22
1003	199	71	21	75	60

India to *H. nodulosum*. According to their description, the conidia were 40–114 × 11–21 μ (mean 67 × 14 μ) and 3- to 11-septate (mean 8-septate). In a compilation of *Helminthosporium* species on grasses, Sprague (21) used Butler's (3) description for *H. nodulosum* because it was the most complete description available under this name; however, it is evident from the descriptions and illustrations in the reports cited that the *H. nodulosum* of Butler and of Mitra and Mehta is entirely different from the type of this species. Their fungus is related to *H. sacchari* and should not be confused with *H. nodulosum*. The fungus on *E. coracana* in Eritrea referred to *H. nodulosum* by Castellani (4) also is definitely not this species, although it may represent a growth form of the *H. nodulosum* of Butler. No conidia were present in a specimen of this fungus sent by Prof. E. Castellani; however, the lesions on the leaves resembled those of spot blotch of barley rather than those produced by *H. nodulosum* on *E. indica*.

Helminthosporium nodulosum var. tritici Patel, Kamat, & Padhye.—A fungus attacking wheat in India was described as a new variety of *H. nodulosum* by Patel, Kamat, and Padhye (16). According to their description, the conidia were thick walled, subcylindric, ovate or rarely obclavate, straight or usually slightly curved, dark brown with hyaline end cells, 3- to 12-septate, and 45–140 × 10–16 μ. The base of the conidium terminated in a conspicuous protruding hilum. The description and the accompanying illustrations are sufficient to indicate that this fungus is not remotely related to *H. nodulosum*. *H. nodulosum* var. *tritici* appears to be identical with *H. halodes* Drechs., which in turn is probably synonymous with *H. rostratum* Drechs.

Helminthosporium leucostylum.—Drechsler (7) described a fungus collected on withered leaves of *Eleusine indica* near Washington, D. C., as a new species, *H. leucostylum*. The type collection of *H. leucostylum* has not been deposited in the National Fungus Collections at Beltsville, and it has been impossible to obtain it elsewhere. It is evident from Drechsler's excellent description and illustrations, however, that his material of *H. leucostylum* was similar to material of *H. nodulosum* collected on leaves in Georgia. The short, hyaline or pale fuligenous conidiophores, which Drechsler considered the outstanding characteristic of *H. leucostylum*, are typical of *H. nodulosum* as it occurs on leaves, especially in the earlier stages of development. A culture labelled *H. leucostylum* (*H. nodulosum* No. 1003) originally isolated by C. L. Lefebvre from leaves of *E. indica* collected by E. A. Hollowell at Takoma Park, Maryland, on September 14, 1939, proved to be identical with cultures of *H. nodulosum* isolated in Georgia. It produced perithecia when mated with compatible strains of *H. nodulosum* and produced typical symptoms on both leaves and inflorescences when inoculated on *E. indica*.

Putterill (17) described a fungus causing leaf stripe and blight, seedling blight, and inflorescence blight of *E. indica* in the Union of South Africa. Although she referred this fungus to *H. leucostylum*, she noted that the conidiophores were swollen at the apex and ultimately became nearly as dark as the conidia. From her description and figures, it is obvious that this fungus was typical of *H. nodulosum*.

Mitra and Mehta (13) reported a similar fungus attacking *E. coracana* in India as *H. leucostylum*. The fungus caused leaf spots, which elongated and coalesced to form stripes, and the leaves became shredded. The inflorescences were infected and became sooty in moist weather. Mitra and Mehta reported successful inoculations on seedlings, leaves, and inflorescences of *E. coracana* and on leaves of *E. indica*, *E. aegyptiaca*, Dest., *Pennisetum typhoideum* L. Rich., *Panicum frumentaceum* Roxb., sorghum, and corn. Only yellowed areas were produced on the inoculated leaves, however, and evidently they did not obtain infection resembling symptoms on *E. coracana* in the field. This fungus appears to be identical morphologically with *H. nodulosum*. Since isolates of *H. nodulosum* from *E. indica* in Georgia failed to infect *E. coracana*, it is possible that the fungus on *E. coracana* in India represents a distinct pathologic race of *H. nodulosum*.

Helminthosporium kusanoi.—Nisikado (14) described a fungus causing blight of *Eragrostis major* Host in Japan as *Ophiobolus kusanoi* Nisikado. The Helminthosporium imperfect stage was not given a specific name at that time, but Nisikado (15) subsequently referred to it as *H. kusanoi*. On *E. major*, *H. kusanoi* produced water-soaked lesions that became grayish brown and elongated until they measured 10–15 × 1 mm; some reached lengths of as much as 30 mm. The lesions coalesced, and the leaves died back from the tips. Almost all inflorescences were infected, and the glumes were covered with a black fungus growth so that they appeared smutted.

The conidia were thick walled, dark olive, short-obclavate or long-elliptical, 25.5–66.3 × 11.5–19.1 μ (mean 43.4 × 14.6 μ), and 1- to 6-septate (mean 3.5-septate). The conidiophores arose singly or in tufts of 2–7 from stomata of dead tissue. They were 25–250 (usually 40–100) × 6.5–7.7 μ, 1- to 5-septate, somewhat swollen at the conidial scars and at the apex, which resembled the head of a peg, and dark olive at the base but becoming paler toward the apex. Although the range of conidial septation was given as 1 to 6, the photographs and drawings show only short obclavate-ellipsoidal conidia with never more than 4 septa.

Although the type collection of *H. kusanoi* could not be obtained, a culture (No. 1023) of this species deposited in the Centraalbureau Voor Schimmelcultures by Nisikado was examined. Conidia produced in this culture (Fig. 10) were nearly identical with those shown in Nisikado's (14) figures. They were elliptical to short-obclavate, thin walled, medium olivaceous-brown, 22.8–48.6 × 9.9–16.7 μ (mean 35.1 × 14.2 μ), and 2- to 4-septate (mean 3.1-septate), with a length/width ratio of 2.5.

Nisikado separated *H. kusanoi* from *H. leucostylum* on the basis of its dark-colored conidiophores. *H. kusanoi* must, however, be compared with *H. nodulo-*

sum in which the conidiophores may become dark colored. Nisikado, who apparently correctly identified *H. nodulosum* on *Eleusine indica* in Japan, stated that the conidia of *H. kusanoi* resembled those of *H. nodulosum* but were shorter. *H. kusanoi* differs in its shorter conidia, never producing the long-obclavate forms typical of *H. nodulosum*, in the lesser number of conidial septa, and in the lesser length/width ratio.

The following 2 North American collections on *Eragrostis* spp. in the National Fungus Collections appear to be referrable to *Helminthosporium kusanoi:*

1) R. Sprague, August 13, 1941, Miles City, Montana, on *Eragrostis cilianensis* (determined as *H. hadrotrichoides*). The specimens consisted of entire plants. The fungus was present on a few dead leaves, and no definite symptoms were evident. The mature seed heads were not infected. Conidia (Fig. 12) were ellipsoid or usually short-obclavate, pale to moderately dark brown, often with a hyaline area surrounding the inserted hilum, 28.9–48.6 × 12.2–17.5 μ (mean 40.0 × 14.3 μ), and 3- to 4-septate (mean 3.5-septate) with a length/width ratio of 2.8. Conidiophores were short, simple, cylindrical, slightly swollen at the tips and at the conidial scars, hyaline to pale brown, 0- to 4-septate, and 30.4–76.0 × 4.6–6.1 μ, the tips measuring 6.1–7.6 μ diameter. (Fig. 11).

2) R. Sprague, September 10, 1940, Ames, Iowa, on *Eragrostis* sp. (determined as *H. rostratum*). The fungus was present on a few dead leaves. There were no definite lesions, and the seed heads were not infected. Conidia (Fig. 11) were ellipsoid to short-obclavate, brown, 25.8–45.6 × 12.2–15.2 μ (mean 36.8 × 13.5 μ), and 1- to 4-septate (mean 3.3-septate). The length/width ratio was 2.7. Conidiophores were short, simple, cylindrical, hyaline to pale brown at the base, slightly swollen at the tips and conidial scars, 3- to 4-septate, and 32.6–83.6 × 4.6–6.1 μ, with swollen tips 6.8–7.6 μ in diameter.

Drechsler (7) reported that he collected *H. leucostylum* repeatedly on *Eragrostis major* in Maryland and Virginia. Stripe lesions were produced on living leaves. Although Drechsler gave no description of this material and deposited no specimens, it is possible that his collections on *E. major* represented *H. kusanoi.*

Nisikado (14) reported that in inoculations with *H. kusanoi* on a series of grasses he obtained infection only on *Eragrostis cilianensis* and *Eleusine indica.* He gave no data on the type of infection resulting from these inoculations. In the present study, inoculations with isolates No. 1060-6 and No. 1177 of *H. nodulosum* and isolates No. 1023 (Nisikado strain from Baarn) and No. 1109 (N.I. 4056, Nagao Institute, Tokyo) of *H. kusanoi* were made on seedlings of *Eleusine indica* and *Eragrostis cilianensis* in the 2-leaf stage. Two 6-in. pots of seedlings of each host were inoculated with each isolate. Both isolates of *H. nodulosum* produced severe systemic infection of Eleusine plants but no infection of Eragrostis plants by the time they reached maturity. Isolates of *H. kusanoi* produced no infection on either host. These results suggest that *H. nodulosum* is restricted to *Eleusine indica*, but the data are weakened by failure to obtain any infection with *H. kusanoi*, which may be attributable to age of these isolates. Cross inoculations with fresh isolates of *H. kusanoi* and *H. nodulosum* would be desirable to determine host specificity in these fungi.

Crosses between isolates of *H. kusanoi* and *H. nodulosum* failed to produce perithecia, although a few tiny black sclerotic bodies were formed when isolates of *H. kusanoi* were mated with either "A" or "a" strains of *H. nodulosum.*

Cochliobolus kusanoi (Nisikado) Nisikado ex Dast.—Nisikado (14) found the perfect stage of *Helminthosporium kusanoi* in the field on dead tissue of *Eragrostis major* and described it as *Ophiobolus kusanoi.* The species was later transferred to the genus *Cochliobolus* by Dastur (6). Conidia of *H. kusanoi* were produced in cultures from single ascospores. Perithecia were produced in a single-conidium culture on rice agar held at 11–15°C for 7–8 weeks. Perithecia were erumpent, black, globose or subglobose with a short beak, and 300–350 μ in diameter. Asci were hyaline, thin walled, cylindric or long-clavate, short-stipitate, 8-spored, 130–170 × 14–18 μ, and interspersed with numerous, hyaline, slender paraphyses 1.5–2 μ in diameter and equalling the asci in length. Ascospores were hyaline, filamentous, thin walled, 140–170 × 5 μ, 6- to 8-septate, and constricted at the septa. They were spirally arranged in 2–3 turns in the ascus.

The type could not be obtained, but the species was adequately described and illustrated.

Helminthosporium hadrotrichoides.—Nisikado (14) suggested the possible identity of *H. kusanoi* with this species, and Sprague (21) reduced *H. leucostylum* to synonymy with *H. hadrotrichoides.* Since the original description of *H. hadrotrichoides* (8) was inadequate, examination of the type was essential.

The type collection (A. Commons No. 347, September 17, 1886, Faulkland, Delaware, on *Eragrostis major*) was obtained from the New York Botanical Garden. The leaves in this collection showed definite lesions in the form of rectangular streaks in the leaf blade that measured 3–25 × 0.8–1.3 mm. The lesions were pale buff to almost white, with fine, indistinct, pale brown borders. They were sometimes coalesced to form long stripes. Conidia (Fig. 8) were ovoid to ovoid-ellipsoid, widest at or slightly below the middle, broadly rounded at the base and tapered to the narrower but still rounded apex, pale to moderately dark brown, 42.0–61.6 × 15.4–22.4 μ (mean 57.0 × 17.8 μ), and consistently 3-septate. The length/width ratio was 2.9. Conidiophores were cylindrical, simple or sometimes short branched toward the apex, swollen at the apex and conidial scars, subhyaline to pale fuligenous, becoming pale fuligenous-brown, and 44.8–126.0 × 5.6–7 μ; the swollen tips measured 9.0–10.6 μ in diameter (Fig. 8).

H. hadrotrichoides, as represented in the type collection, is distinguished from collections of *H. kusanoi* by the broader, consistently 3-septate conidia and the more conspicuously swollen conidiophore tips.

The following additional collections, which may rep-

resent *Helminthosporium hadrotrichoides*, have been examined:

1) New York Botanical Garden. A. Commons, September 1887, Faulkland, Delaware, on leaves of *Eragrostis major*. This collection evidently was from the type locality and was nearly identical with the type. Conidia were obclavate-ellipsoid, pale to moderately dark brown, 42.0–58.8 × 14.0–21.0 μ (mean 51.0 × 17.5 μ), and consistently 3-septate. Conidiophores measured 61.6–117.6 × 5.0–5.6 μ and 9.8 μ in diameter at the swollen tips.

2) National Fungus Collections. H. C. Greene, August 11, 1947, Madison, Wisconsin, on *Eragrostis cilianensis* (determined as *H. rostratum*). The collection contained a few leaves in the early stages of infection; these showed elliptical grayish brown spots with very narrow brown borders measuring 10–12 × 1–2.5 mm. Conidia were ellipsoid to obclavate, pale olivaceous brown, 27.4–59.3 × 12.9–16.7 μ (mean 43.8 × 14.5 μ), and 2- to 3-septate (almost constantly 3-septate) with a length/width ratio of 3.0 (Fig. 9). Conidiophores were very short, simple, hyaline to pale brown, 1- or 2-septate, 25.8–50.2 × 5.8–6.1 μ, and 7.6–9.9 μ in diameter at the swollen tips (Fig. 9). This collection differs from the type collection of *H. hadrotrichoides* in its narrower condia, which may not have been fully mature.

Separation of species.—*Helminthosporium nodulosum*, *H. leucostylum*, *H. kusanoi*, and *H. hadrotrichoides* form a closely related group distinguished from other species of *Helminthosporium* by the relatively small, few-septate, characteristically obclavate, brown, thin-walled conidia and the peculiar conidiophores, which are swollen at the tips and nodes and in the early stages are hyaline throughout, although they may become darker toward the base and on seed heads may become long, occasionally branched, and as dark as the conidia. The species parasitizing *Eleusine* spp., *H. nodulosum* and *H. leucostylum*, are identical, the types of each merely representing growth forms of the same fungus. This is demonstrated by the production of both foliage lesions bearing material typical of that on which *H. leucostylum* was based and sooty heads of the inflorescence as in the type collection of *H. nodulosum* on inoculation with single conidium isolates from either leaves or seed heads.

The species described on *Eragrostis* spp., although closely related to *H. nodulosum*, differ from it, in addition to hosts, in their shorter fewer-septate conidia. In the few available collections on *Eragrostis* spp., there appear to be 2 distinct groups of fungi referrable, respectively, to *H. kusanoi* and *H. hadrotrichoides*. They may be separated on the basis of conidial size and septation and the degree of swelling of the conidiophore apex. Further study of more extensive collections on *Eragrostis* spp. may demonstrate that *H. kusanoi* should be considered a synonym of *H. hadrotrichoides*, and both may prove to be forms of *H. nodulosum*. On the basis of present evidence, they are tentatively maintained as distinct species. They may be separated according to the following key:

KEY TO HELMINTHOSPORIUM NODULOSUM AND RELATED SPECIES

Conidia 1- to 6(7)-septate, obclavate; conidiophores simple or occasionally branched, hyaline in the early stages, with swollen, pestle-shaped tips.

A. Conidia 3- to 7-septate, averaging 5 or more septa, long obclavate, length/width ratio 3.5 or more; conidiophore tip prominently swollen, 6.4–9.0 μ diameter; on *Eleusine* spp. ..... *H. nodulosum*

AA. Conidia 1- to 4(6)-septate, averaging less than 4 septa, short obclavate to ovate, length/width ratio 3.0 or less; on *Eragrostis* spp.

B. Conidia consistently 3-septate; conidiophore tip prominently swollen, 7.6–10.6 μ diameter ..... *H. hadrotrichoides*

BB. Conidia 1- to 4(6)-septate; conidiophore tip less prominently swollen, 6.1–7.6 μ diameter ..... *H. kusanoi*

The perfect stage of *Helminthosporium nodulosum* is nearly identical with *Cochliobolus kusanoi* Nisikado (14). Both are characterized by perithecia, asci, and ascospores that are smaller than in any other species except *C. sporoboli* Castell. Dimensions given for *C. sporoboli* (5) are even smaller than those recorded for *C. kusanoi*. Since the conidial stages are distinct, the perfect stage of *H. nodulosum* is described as a new species of *Cochliobolus* as follows:

Cochliobolus nodulosus sp. nov.

Peritheciis superficialibus, nigris, globosis, 276–414 μ diam., in papillatum 97–262 × 55–83 μ abeuntibus, carbonaceis, contextu pseudoparenchymatico; ascis 1- ad 8-sporis, cylindraceis, rectis, supra rotundatis, basi breve pedicellatis, unitunicatis, 120–193 × 14.0–16.8 μ, pseudoparaphysatis; ascosporis hyalinis, filiformibus, utrinque acutatis, media parte saepe leniter nodulosis, 90–193 × 3.9–7.8 μ, 3- ad 11-septatis, spiraliter convolutis.

Status conidicus: Helminthosporium nodulosum Berk. et Curt. ex Sacc. Habitat: In culturis in stramine Hordei vulgaris et in fructibus Zeae maydis.

Perithecia superficial, black, spherical, 276–414 μ diam., with long cylindrical beaks 97–262 × 55–83 μ. Asci unitunicate, 1- to 8-spored, cylindrical, straight, rounded at the apex, short-stipitate, 120–193 × 14.0–16.8 μ, interspersed with pseudoparaphyses. Ascospores hyaline, filiform, tapering slightly toward either end, spirally coiled within the ascus, 120–193 × 14.0–16.8 μ, 3- to 11-septate, more or less deeply constricted at the septa in the middle portion, germinating from any or all cells.

Conidial stage: *Helminthosporium nodulosum* Berk. & Curt. ex Sacc. Developed in culture on sterilized fragments of barley straw and on corn grains in matings of compatible strains.

Specimens have been deposited in The National Fungus Collections, Beltsville, Maryland, and The Herbarium of The Royal Botanic Garden, Kew.

DISCUSSION.—Systemic infection in the genus *Helminthosporium* has been demonstrated previously only in *H. gramineum* Rabh. (20), a species included in the subgenus *Cylindro-Helminthosporium*. *H. nodulosum* is the first species in *Euhelminthosporium* in which systemic infection has been found. The relationship between *H. nodulosum* and plants of *Eleusine indica* apparently is not well balanced, since the majority of the infected plants die in the seedling stage. More tolerant varieties of *E. indica* might be developed through breeding. Differences in virulence among isolates of *H. nodulosum* apparently have little effect on

the parasite-host balance. Strains of the fungus that caused little killing of seedlings also produced few infections, and the percentages of plants infected and surviving to maturity were not greater than with more virulent strains. A similar type of systemic infection of *Phragmites communis* Trin. by *Napicladium arundinis* (Cda.) Sacc. has been observed (9), although it has not been demonstrated experimentally.

Helminthosporium nodulosum is unusual among systemic fungi in that infection, although limited to immature tissue, may occur at any stage in the development of the host until it approaches full maturity. When infection of nearly mature plants occurs, the fungus may be limited to the uppermost leaves that were immature at the time of infection. Local lesions may thus be produced, although infection is basically systemic in nature. Systemic infection of sorghum plants as much as 9 weeks old with *Sphacelotheca reiliana* (Kühn) Clint. may result from transferring seedlings to contaminated soil (10), and infection of older sorghum and Johnson grass plants with *Sphacelotheca cruenta* (Kühn) Potter may be obtained by applying spores to the cut surface of stubble (11). These cases, however, are not strictly comparable. Infection of individual tillers of *Eleusine indica* by *H. nodulosum* also may result in partially affected plants. Similarly, smutting of the heads on secondary branches of sorghum by *Sphacelotheca sorghi* (Lk.) Clint. may occur, but Melchers (12) attributed this to latent infection.

Helminthosporium nodulosum is the second species in the subgenus *Euhelminthosporium* in which perithecia have been produced in culture on a reproducible basis by mating strains of opposite sex-reaction type. This phenomenon was first reported in *H. sorokinianum* Sacc. ex Sorok. (*Cochliobolus sativus* (Ito & Kuribay.) Drechs. ex Dast.) by Tinline (22). The fact that the methods employed for production of perithecia in *H. sorokinianum* (19, 22) have proved effective with the rather distantly related *H. nodulosum* suggests that the perfect stages of other species in *Euhelminthosporium* may be obtained if an extensive search is made for compatible strains. The problem may be more difficult in other species than in *H. nodulosum*, in which strains isolated from the field were approximately equally divided between the 2 sex-reaction groups. Aside from other considerations, results of genetic studies and interspecific matings would be of great value in determining taxonomic relationships among species of *Helminthosporium*.

Nisikado (14) reported that perithecia were produced in a single-conidium culture of *H. kusanoi*. Unfortunately, he did not mention attempting to obtain perithecia in single-ascospore cultures. It is doubtful that *H. kusanoi* differs from the closely related *H. nodulosum* in being self-fertile. It is possible that Nisikado's results may be explained on the basis of heterocaryosis or contamination.

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PECTOLYTIC ENZYMES SECRETED BY SOFT-ROT BACTERIA[1]

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SUMMARY

Erwinia carotovora, *E. atroseptica*, and *E. aroideae* produced no pectin methyl esterase and no polygalacturonase but an abundance of depolymerase on synthetic medium containing pectin. The macerating activity of the enzymes of *E. carotovora* and of *E. atroseptica* was greatest at pH 6.5; that of *E. aroideae* was greatest at pH 8.9. Activity of the enzymes of all 3 organisms was reduced when they were heated at progressively higher temperatures, for a 10-minute interval, up to 65° C. There was slight activity in each case after boiling. Enzyme production of *E. aroideae* and *E. carotovora* was greatest in media adjusted to pH 7–9, and the enzymes were more active on pectate than on partially demethylated pectin substrate.

The pectolytic enzyme produced by *Erwinia aroideae* (Towns.) Holland was studied by Wood (10). He found that this organism produced little or no pectin methylesterase (PME) on synthetic medium. The enzyme preparation reduced the viscosity of pectin and pectate solutions but failed to reduce the substances to monogalacturonic acid as determined by paper chromatography. It was concluded that the hydrolytic enzyme concerned was a pectic depolymerase (DP). The enzyme activity was optimum at pH 9. The activity of the enzyme was reduced to a low level at 65° C.

The present study is concerned with a similar study of *Erwinia carotovora* (L. R. Jones) Holland and *E. atroseptica* (v. Hall) Jennison, in comparison with *E. aroideae*.

CHARACTERIZATION OF THE ORGANISMS USED.—The isolate of *E. carotovora* used was obtained from W. H. Burkholder of Cornell University, and the isolate of *E. aroideae* used was obtained from P. A. Ark of the University of California. Three single-cell subcultures of each isolate were compared on differential media. All produced narrow infundibuliform growth on nutrient bacto-gelatin at 20° C after 4 days. None produced indole on tryptone broth. All produced nitrite on synthetic medium containing nitrate. The subcultures of *E. aroideae* produced H_2S abundantly whereas those of *E. carotovora* produced little or none. Gas formation and acid production were studied on casein-hydrolysate peptone broth containing 1 per cent glucose, lactose, xylose, maltose, sodium succinate, sodium malonate, glycerol, or starch. Acid was formed in all cases. No gas was produced by *E. aroideae* on any of the media; slight gas formation occurred with *E. carotovora* on media containing glucose, lactose, or xylose. *E. aroideae* grew well at 40° whereas *E. carotovora* showed slight growth at this temperature. The culture designated as *E. aroideae* was regarded as a valid member of that species because of its production of hydrogen sulfide, its strong growth at 40°, and its complete lack of gas-forming ability on carbohydrate substrates. This is in accord with the differences between the 2 species emphasized by Holdeman and Burkholder (5). Likewise, according to the tests indicated, the culture designated as *E. carotovora* was considered a valid member of that species.

Two isolates were secured from potato plants showing typical black-leg symptoms in central Wisconsin in 1956. These isolates, together with the above-mentioned isolates of *E. carotovora* and *E. aroideae*, were used to inoculate stems of young potato plants in the greenhouse at 22° C. Bacteria from the respective cultures were inserted into stems just above the soil line by means of needle punctures, and moist vermiculite was placed around the wounds. Typical black-leg symptoms resulted from inoculations with the 2 isolates from the potatoes affected by black leg, but none occurred in plants inoculated with either of the other 2 isolates. *E. carotovora* and the isolates from black-leg-affected potato were compared on casein-hydrolysate peptone broth containing 1 per cent glucose, lactose, xylose, or sodium malonate or 5 per cent ethanol. Gas formation and acid production took place on all the media seeded with *E. carotovora*. The isolates that incited black leg produced acid and gas on media containing glucose, lactose, or xylose, but neither was produced on media containing sodium malonate or ethanol. This is in accord with the report of Burkholder and Smith (2), who pointed out these distinctions between the 2 species. On the basis of the cultural and pathogenicity tests, the 2 isolates from potatoes affected with black leg appeared to be identical and both conformed to the characteristics of *E. atroseptica*. One of the isolates was used in further study.

PREPARATION OF PURIFIED ENZYMES.—In the early part of the investigation a modification of Fernando's (4) medium was sued. This consisted of $MgSO_4 \cdot 7H_2O$, 5 g; KH_2PO_4, 6.8 g; asparagine, 5 g; commercial pectin, 1 g; glucose, 15 g; distilled water, 1000 ml. This medium was adjusted to pH 7 with 0.1N NaOH after autoclaving. It was found later that the enzyme yield was greater when use was made of the following modification: $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 2.0 g; asparagine, 2.5 g; commercial pectin, 1 g; sucrose, 10 g; distilled water, 1000 ml. This medium had a reaction of pH 7 without further adjustment. Cultures were grown in 1-liter flasks for 3 or 4 days on a mechanical shaker. The cultures were filtered through a Büchner funnel, and 1 liter of filtrate

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was placed in a vacuum still where the temperature did not exceed 30° C. When the volume was reduced to 300 ml, 4 volumes of ethanol was added slowly with mechanical agitation; the mixture then was stored at 4° for 24 hours. The precipitate was removed by centrifugation, dried over calcium chloride, and stored at 4° until used. When the preparation was to be used, it was suspended in distilled water and brought up to the original volume.

DETERMINATION OF ENZYMES PRESENT.—Pectin methyl esterase (PME) activity was investigated by the method described by Winstead and Walker (9). The reaction mixture was adjusted to pH 6.5 with McIlvaine's (1, p. 1615) buffer. No evidence of PME was obtained in culture filtrates from each of the 3 species. This is not in accord with the report of Kraght and Starr (6).

When enzyme preparation solutions from the 3 organisms were added to 1.5 per cent pectin solution or to 0.5 per cent sodium polypectate solution and the viscosity was determined by means of an Oswald viscosimeter, there was a decided drop in viscosity with time, indicating the presence of pectin depolymerase (DP) and/or polygalacturonase (PG). A mixture of 10 ml of each enzyme preparation and 25 ml of 0.5 per cent sodium polypectate was allowed to digest, and samples were taken at 0, 24, and 48 hours for analysis by ascending paper chromatography. Whatman No. 1 filter paper was used with a solvent system containing 1-butanol, acetic acid, and water (4:1:5). A solution of monogalacturonic acid was used as a control. When the solutions had risen to about 1 in. from the top, the paper was removed and sprayed with a solution made up of 0.5 g benzedine, 10 ml acetic acid, 10 ml 45 per cent trichloroacetic acid, and 100 ml 95 per cent ethanol. It was next placed in an oven at 90° C for 2 minutes and then examined. The monogalacturonic acid gave a distinct pink spot. No corresponding spots were observed in tests with the enzyme samples. Since there was no evidence of monogalacturonic acid, it was concluded that the hydrolytic enzymes present belonged to the DP group as already reported for *E. aroideae* by Wood (10).

RELATION OF pH TO ENZYME ACTIVITY AND PRODUCTION.—Blocks 1 cm square of carrot root and of potato tuber were cut on a microtome to 200μ thickness. They were sterilized in a 0.5 per cent solution of sodium hypochlorite and rinsed in sterile distilled water. Ten ml of the enzyme preparation of the crude filtrate from cultures and 5 ml of buffer solution were mixed and the tissue squares inserted. The end point of activity was determined as the number of minutes that elapsed until the block could not be lifted by the edge with forceps. Relative maceration activity was expressed in a given series by giving a value of 100 to the time required for complete maceration by the most active sample. For each of the other samples, the time of the minimum-time sample was divided by the time of the sample in question and the quotient multiplied by 100. The enzyme preparation and the culture filtrate of each organism were tested at several pH levels. Maceration of carrot tissue discs at the optimum pH occurred in 30–45 minutes with *E. carotovora* and *E. atroseptica* and in 60–75 minutes with *E. aroideae*. For each organism, culture filtrates and purified enzyme preparations gave similar results. The data from a representative experiment are given in Table 1. The optimum activities of the enzymes of *E. carotovora* and of *E. atroseptica* were at pH 6.5. In this test the greatest activity of the enzyme of *E. aroideae* was at the highest pH used (pH 8.5). In another test, the activity increased with increase in pH up to pH 8.9 and was reduced slightly at pH 9.4. This is close to the optimum (pH 9) given by Wood (10) for this organism. When the enzymes of *E. aroideae* and *E. carotovora* were compared as to maceration activity on potato discs, the respective optima were again pH 8.9 and pH 6.5.

TABLE 1.—*Maceration activity on carrot tissue discs of filtrates from cultures of Erwinia aroideae, E. carotovora, and E. atroseptica, when filtrates were adjusted to various pH levels*

pH	Relative activity on substrate by culture filtrate indicated [a]		
	E. aroideae	*E. carotovora*	*E. atroseptica*
5.0	25	84	76
6.0	40	90	87
6.5	62	100	100
7.0	71	90	87
8.0	80	60	63
8.5	100	45	50

[a] In each column, the value for the most rapid maceration test was set at 100. For each other test, the time required for the most rapid sample was divided by the time of the sample in question and the quotient multiplied by 100 to obtain the relative activity value.

The effect of pH on enzyme activity was determined next by rate of reduction of viscosity of a pectate solution. Two ml of enzyme-precipitate solution was added to 10 ml of 0.5 per cent sodium polypectate solution buffered to the desired pH with 2 ml of the appropriate McIlvaine's buffer solution. The viscosity of each preparation was determined at 15 minutes by means of an Oswald viscosimeter. The optimum pH for viscosity reduction by each respective organism was similar to that obtained in the maceration test.

E. aroideae and *E. carotovora* were grown on Fernando's medium at several pH levels for 3 days. Five-ml portions of the filtrate were tested for reduction in viscosity as a measure of the amount of enzyme present. Both organisms reached maximum enzyme production at pH 7 and continued to produce at that level up to pH 8.6 or pH 8.8 (Table 2). Thus there was no correlation between pH optimum of enzyme production and that of enzyme activity.

ENZYME ACTIVITY ON PECTIN AND PECTATE SUBSTRATES.—The activities of the enzyme preparations from *E. aroideae* and *E. carotovora* were compared on substrates containing pectin and on those containing sodium polypectate. The commercial pectin used had about 9 per cent methylation compared with approximately 15 per cent in a fully methylated preparation. Two ml of enzyme-preparation solution was added to

TABLE 2.—*Enzyme activity, as determined by reduction in viscosity of pectate solution, of filtrates of cultures of Erwinia carotovora and E. aroideae after 3 days' growth at different pH levels in Fernando's medium*

E. carotovora			*E. aroideae*		
pH of medium		Percentage reduction in viscosity	pH of medium		Percentage reduction in viscosity
Initial	Final		Initial	Final	
4.0	4.0	0	4.0	4.0	0
5.0	5.0	0	5.0	5.0	0
6.0	6.0	53.1	5.9	6.6	90.9
7.0	8.2	97.6	6.9	8.2	97.9
8.0	8.4	97.6	7.7	8.7	98.3
8.6	7.8	96.5	8.8	7.7	95.6

10 ml of 0.5 per cent solution of sodium polypectate or of pectin. The mixtures of the enzymes of *E. carotovora* were adjusted to the pH optimum for enzyme activity (pH 6.5). Those of *E. aroideae* were adjusted to pH 7, since pectin tends to de-esterify spontaneously at pH 9, the optimum for the enzyme concerned. The results of viscosity determinations made at various intervals are presented in Figure 1. In the case of the enzymes from each organism, the reduction of viscosity was more rapid on pectate than on pectin substrate. These results are in accord with those of Scheffer et al (8) with *Verticillium albo-atrum* Reinke & Berth., the culture fluids of which showed the presence of polygalacturonase (PG) and very little PME. The culture solution was very active on pectate substrate, it was less so on 9 per cent methylated commercial pectin, and least active on fully methylated pectin. In another study, Echandi and Walker (3) showed that the culture filtrate from *Sclerotinia sclerotiorum* (Lib.) d By., which contained both PME and PG, was about equally active on pectate and commercial pectin substrates. It would appear, therefore, that those organisms that produce PG and/or DP but little or no PME are most active on pectate whereas those which also produce PME are more nearly equal in activity on pectin and on pectate.

RESISTANCE OF THE ENZYMES TO HEAT.—Wood (10) noted a progressive decline in activity of the enzyme of *E. aroideae* with increases in temperature up to 65° C, at which point about 5 per cent of the original activity was evident. In this investigation, enzyme preparation solutions from the 3 organisms were exposed to various temperatures for 10 minutes, then they were cooled to room temperature and the percentage reduction in viscosity determined. The preparations from the 3 organisms responded similarly to heat, dropping in activity when heated at progressively higher temperatures; after treatment at 65°, the activity was about 15 per cent of the original. There was very slight activity, however, in each of the 3 preparations after they had been boiled for 10 minutes. McColloch and Kertesz (7) reported that an enzyme preparation from tomato fruit differed from a pectolytic complex prepared from fungi in that the former retained about 10 per cent of its original hydrolytic activity after it had been boiled for 10 minutes.

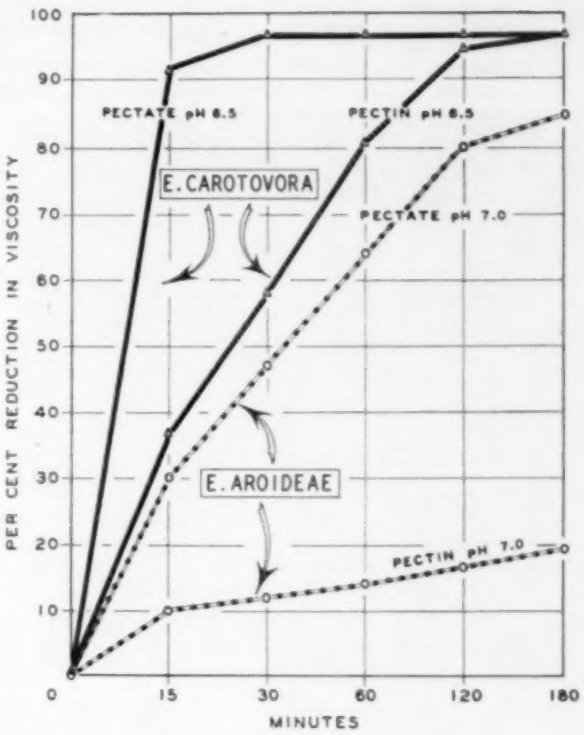

FIG. 1. Hydrolytic activity of depolymerase when prepared from cultures of *Erwinia carotovora* and *E. aroideae* and tested on pectin and on sodium polypectate substrate. Activity was measured as percentage reduction in viscosity over a period of 180 minutes.

DISCUSSION.—The 3 common soft-rot bacteria were found to be similar in that they produced only depolymerase on synthetic medium containing pectin. They were also similar in their response to increase in temperature; however, the enzymes of *E. carotovora* and of *E. atroseptica* differed markedly from that of *E. aroideae* in the optimum pH for activity. Comparable preparations of the enzymes of *E. carotovora* and of *E. atroseptica* caused more rapid maceration of carrot tissue than did that of *E. aroideae* at their respective optimum pH levels. Inasmuch as the deploymerases produced by *E. carotovora* and *E. atroseptica* were essentially alike, there was no indication that the property of inciting black leg of potato confined to the latter species was associated with pectolytic activity.

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THE HOMEGARDEN CANTALOUPE, A VARIETY WITH COMBINED RESISTANCE TO DOWNY MILDEW, POWDERY MILDEW, AND APHIDS [1]

S. S. Ivanoff

SUMMARY

A new cantaloupe, named Homegarden, is a progeny of a cross between a downy-mildew and aphid-resistant inbred of Smith's Perfect and Powdery Mildew Resistant California No. 5. Homegarden has moderate field resistance to downy mildew (caused by *Psuedoperonospora cubensis* (Berk. & Curt.) Rostow) and to powdery mildew (caused by *Erysiphe cichoracearum* DC.) and high resistance to the melon aphid (*Aphis gossypii* Glover). The vine is vigorous and medium large. The fruit is globose and of medium size, with scant netting, small blossom end, and thin rind. The flesh is orange and thick; it is hard at first, later becoming tender. The fruit has a tendency to crack, particularly in wet weather. The seed cavity is small. The flavor is distinctive, and sugar and vitamin C contents are very high. It is early to midseason in maturity.

A new cantaloupe (Fig. 1) of high quality, named Homegarden, has been developed. It has resistance to downy mildew (caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow), to powdery mildew (caused by *Erysiphe cichoracearum* DC.), and to the melon aphid (*Aphis gossypii* Glover). It originated from a cross between an aphid-resistant inbred selection of Smith's Perfect and California Powdery Mildew Resistant No. 5 (PMR5). Many selections were made from this cross for several generations, and each selection was tested for disease and aphid resistance under artificial and/or natural conditions. Particular attention was given to the epicurean qualities of the fruit, since the most important consideration in choosing a cantaloupe is its taste. Disease and insect resistance are an aid to quality, and this is particularly true for cantaloupes.

DESCRIPTION.—As the name indicates, Homegarden is intended for home growing and is not suitable for shipping. It is early to midseason. The vine is medium large and vigorous. The fruit is of medium size and globose, with scant netting. Shade increases the netting, whereas exposure to the sun produces sleek melons of lower quality. The blossom end is small and the rind thin; the flesh is orange and thick, hard at first, later becoming tender; the cavity is small. The fruit has the tendency to crack, particularly in wet weather. It may be harvested "full slip" before it is ready to eat.

RESISTANCE TO DOWNY MILDEW AND APHIDS.—These characteristics were inherited from Smith's Perfect, which was not genetically stable when first obtained. This variety was inbred for 4 generations, and the most resistant inbred selection was then used in the crossing. It has been previously reported that in such cantaloupes as Rocky Dew Green Flesh, Rocky Dew Orange Flesh, Cuban Castillian, and Smith's Perfect, downy mildew resistance is closely associated with aphid resistance.[2, 3] This fact has facilitated the breeding for combined resistance, since it was only necessary to test aphid resistance, for which an artificial, quick, and accurate seedling method has been developed.[4] The aphid-resistant young seedlings thus selected usually proved resistant to downy mildew also. This particular association pertains only to the above-named varieties of cantaloupes and to no other varieties or species of plants as far as known to this writer. The aphid resistance of the new cantaloupe is of a high order (Fig. 2, A).

RESISTANCE TO POWDERY MILDEW.—The powdery mildew resistance of Homegarden was inherited from PMR 5. Its resistance, however, is lower than that of the parent; in the greenhouse in the presence of abun-

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Mississippi Agricultural Experiment Station, Journal Paper (New Series) No. 648.

[2] All 4 varieties have been introduced into the United States from the West Indies, although originally they were brought in from Spain.

[3] Ivanoff, S. S. 1944. Resistance of cantaloupes to downy mildew and the melon aphid. J. Heredity 35: 34-39.

[4] Ivanoff, S. S. 1945. A seedling method for testing aphid resistance. J. Heredity 36: 357-361.

dant inoculum, Homegarden has been subject to considerable infection. The disease begins to become serious, however, only after susceptible varieties, such as Hale's Best, have been destroyed by mildew (Fig. 2, B). Its field resistance, in contrast, is sufficient in most cases. No powdery mildew has been noted in the

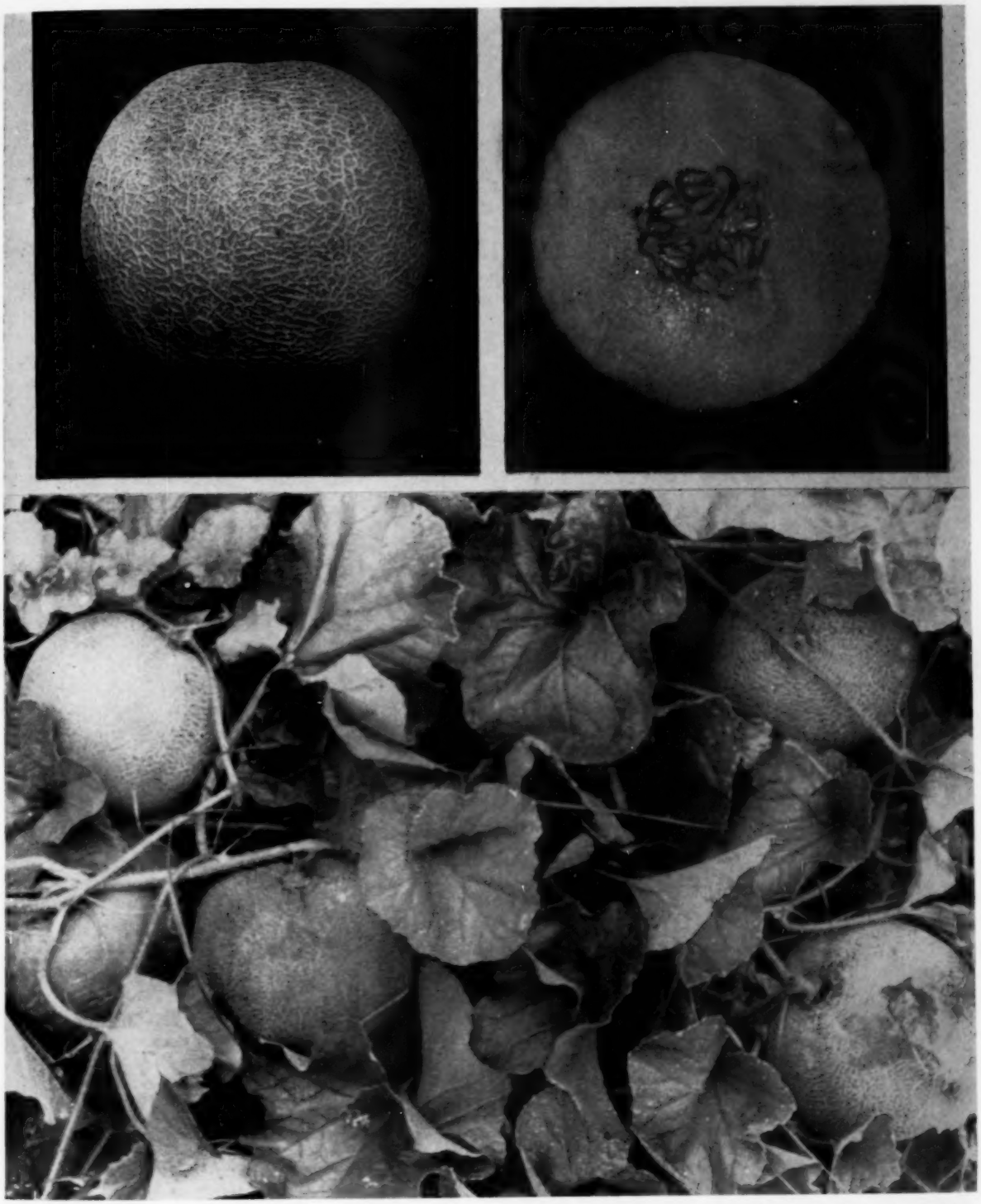

FIG. 1. View of Homegarden cantaloupe fruits. Lower picture shows part of a single vine that had 7 fruits, 5 of which are shown. Under certain conditions, the vines will produce a second crop of melons after the first crop has been harvested.

FIG. 2. A) Homegarden and Hale's Best cantaloupe varieties compared for aphid and powdery mildew resistance. Left, Homegarden without any aphids; right, Hale's Best killed at an early stage by aphids. B) Representative leaves of 3 cantaloupe varieties exposed to powdery mildew in the greenhouse: Hale's Best, Homegarden, and Smith's Perfect. Homegarden showed on the average less than 1 mildew lesion per leaf (plants about 8 weeks old). California Powdery Mildew Resistant No. 5 (not shown) did not exhibit any mildew.

FIG. 3. Reaction to aphid infestation of the 2 parent cantaloupe varieties and the F-1 cross from which Homegarden originated. A, D) Smith's Perfect aphid-resistant inbred parent. No curling of leaves. B) Aphid-susceptible California PMR 5. Note curling and shrivelling of leaves on account of aphids. C) The F-1 generation, Smith's Perfect × PMR 5. Aphid-resistant, no curling of leaves. E) Hale's Best (strain Seed Breeders) grown as aphid-susceptible check. Some powdery mildew developed on A and D, none developed on B and C, and it was severe on E (photograph taken 33 days after planting).

field on this new variety in Mississippi, although it has occurred on other varieties in severe form.

It is possible that some slight resistance to powdery mildew may also have been inherited from the Smith's Perfect selection. This variety seems to withstand the disease slightly better than do other susceptible commercial varieties. The resistance of Smith's Perfect, however, hardly could be of any practical significance where powdery mildew is a major hazard.

The reactions of the 2 parents and the F_1 generation to aphids were tested simultaneously by the method already mentioned (Fig. 3). As formerly reported, aphid resistance is inherited in a dominant manner and determined by a single gene pair, the F_2 ratio between resistant and susceptible plants being 3:1.[4]

RESISTANCE TO OTHER PESTS.—Some resistance to the melon worm (*Diaphania* spp.) has been noted.

YIELDS.—Homegarden yields well and surpasses many common varieties even in the absence of disease or insect hazards. In 5 field trial plantings, 3 of which suffered disease and/or insect damage, the average yield of graded cantaloupes per acre for the Homegarden was 16,380 lb., against 7400 lb. for Hale's Best, and 8100 lb. for California Powdery Mildew Resistant No. 4-50 and PMR 5. In the 3 seasons mentioned, the commercial varieties produced only a few edible fruits.

FRUIT QUALITIES AND GROWERS' AND CONSUMERS' ACCEPTANCE.—The most important features of this cantaloupe, as far as the consumer is concerned, are its flavor and high sugar content. It has averaged between 12 and 13 per cent total soluble solids (mostly sugars) in 10 field trials made at various places. The soluble solids for some individual fruits have reached as high as 16.5–16.8 per cent. These values are the highest recorded for any cantaloupe variety or strain tested by this writer. No doubt the resistance of the foliage to the mildews and aphids accounts in part for the high quality of the fruit; however, even in the absence of any hazards on the susceptible check varieties, the percentage total solids of Homegarden has been higher than that of others. This cantaloupe has a quality of its own: a blend of spice, sweetness, and aroma that seems to appeal to most people.

Homegarden has been compared for taste by 97 growers with 1 or more varieties of their own choice, grown side by side. The varieties used in these comparisons have been Hale's Best, Rocky Ford, Eden Gem, Rio Sweet, Rio Gold, Texas Resistant No. 1, New Orleans Market, Burpee Hybrid, Honey Rock, Honey Dew, California 45, Persian, Banana, and many others, with Rocky Ford and Hale's Best being most frequently employed. Ninety of the 97 growers preferred Homegarden to the other variety or varieties tasted. Of the 26 growers who compared it with Rocky Ford in particular, 22 liked the taste of Homegarden better than that of Rocky Ford. Of the 21 growers who compared it with Hale's Best, all preferred Homegarden.

The vitamin C content (ascorbic acid) of a large number of varieties and strains, including Homegarden, has been tested by the U.S.D.A. Vegetable Breeding Laboratory at Charleston, S. C. The following is the Laboratory's report. "A study of the ascorbic acid content was begun this year. Approximately 1,000 fruit from the 1956 crop were analyzed. The range of ascorbic acid content of individual fruit was 10.0 to 71.5 mg per 100 grams, fresh weight basis. The fruit which contained the largest amount of ascorbic acid was one of the Homegarden variety. The mean con-

TABLE 1.—*The performance of Homegarden cantaloupe in regard to yield, disease resistance, and insect resistance in comparison with other cantaloupes*

Date of trial	Location in Mississippi	Chief hazards	Varieties compared [a]	Yield (lb./acre) Graded	Yield (lb./acre) Total	Percentage total solids	Downy mildew [b]	Powdery mildew [b]	Aphids [b]
1951 (Spring)	Poplarville	Downy mildew	Homegarden			10.8	None–tr.	None	None
			Hale's Best			7.7	Med.–sv.	None	Trace
			PMR 5			7.9	Med.–sv.	None	Trace
1951 (Fall)	State College	Aphids	Homegarden	12,240	14,760	13.0	None	None	None
			Hale's Best	0			None	None	V. sv.
			PMR 45	0			None	None	V. sv.
			Ga. 47	0			None	None	V. sv.
1951 (Fall)	Parchman	Aphids, downy mildew, melonworms	Homegarden [c]	(High yield)		12.2	None	None	None
			Rocky Ford [d]	(Very low yield)			Med.–sv.	None	Sv.
1952 (Spring)	Oktibbeha Co.	None	Homegarden	20,160	20,880	11.9	None	None	None
			Hale's Best	16,560	18,720	10.0	None	None	None
			PMR 5	17,280	18,080	9.3	None	None	None
1952 (Fall)	State College	Downy and powdery mildews, aphids	Homegarden	16,920	18,000	12.8	Trace	None	Slight
			Hale's Best	360	5,040	7.0	Severe	Sl.–med.	Sv.
			PMR 5	1,440	5,760	6.9	Severe	None	Sv.
1953 (Spring)	Oktibbeha Co.	None	Homegarden	16,560	17,280	11.8	None	None	None
			Hale's Best	13,680	14,760	10.4	None	None	None
			PMR 5	12,960	15,480	10.6	None	None	None
1953 (Fall)	State College	Aphids Downy mildew	Homegarden	11,880	15,120	13.3	None–tr.	None	Tr.–sl.
			Hale's Best	0	1,080	6.7	Med.	Trace	Sv.
			PMR 4-50	720	1,440	6.9	Med.	None	Sv.
	Average (5 trials)		Homegarden	16,380	17,820	12.2			
			Hale's Best	7,400	9,900	8.0			
			PMR 45, PMR 5, PMR 4-50	8,100	10,190	8.0			

[a] PMR represents Powdery Mildew Resistant.
[b] Abbreviations: Med.—medium; sl.—slight; sv.—severe; v. sv.—very severe; tr.—trace.
[c] Homegarden suffered less from melonworms (*Diaphania* spp.) than did Rocky Ford and other commercial varieties.
[d] Grown on same farm but not adjacent to Homegarden.

tent of 17 fruits of this variety, 48.3 mg, was second highest of the 150 lines represented by more than 1 fruit. The highest, which contained 54.2 mg, was a hybrid (Homegarden × Healy's Pride)."[5]

[5] From the 17th Annual Report of Vegetable Breeding in Southeastern U. S., Hawaii and Puerto Rico. Southeastern Vegetable Breeding Laboratory, Agriculture Research Service, U.S. Dept. Agr., 1956 (mimeographed). Unpublished results reported here by permission of the Laboratory.

ADAPTATION.—This new variety was tested in the spring and summer of 1956 by a large number of Mississippi growers, and at some places in Alabama, Arkansas, Louisiana, Massachusetts, California, and other locations, mostly with favorable results.

Seed from commercial sources will become available in the fall of 1957.

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EFFECT OF INSECTICIDE TREATMENTS OF BEETS ON TRANSMISSION OF YELLOWS VIRUS BY MYZUS PERSICAE[1]

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SUMMARY

Viruliferous aphids permitted 20-minute test feeding periods in successive transfers to insecticide-treated plants were not killed until they had fed 80–100 minutes. Some aphids maintained ability to transmit beet yellows virus until they were inactivated. The systemics Thimet and demeton killed feeding aphids for 18–24 days after application. Metacide had a greater residual action (12–16 days) than did parathion (8–14 days) or malathion (6–10 days). Thimet 44-D applied as a seed treatment was not lethal to aphids 21 days after application. Thimet and demeton were translocated from sprayed to unsprayed leaves within 18–24 hours after application. Lethal amounts of parathion, Metacide, or malathion were not translocated.

Virus yellows is an important disease of table beet seed plants (*Beta vulgaris* L.) in the Puget Sound region (5). It has been reported also on sugar beets in the United States (1) and in Europe (2,4), with losses being most pronounced in plants grown for seed. The causal virus is persistent in green peach aphids (6,7). Prevention of spread of the virus in the field involves control of this vector or the growing of stecklings for seed crops in areas free from viruliferous aphids (4, 5). It has become increasingly difficult, however, to find areas free from alate, migratory, viruliferous aphids; in spite of insecticidal applications, considerable virus inoculation has occurred.

In a search for an explanation of the ineffectiveness of insecticide applications, greenhouse experiments were designed to determine 1) the rapidity of lethal action on green peach aphids of certain residual and systemic insecticides applied to beets and the effect of this action on virus transmission, 2) the duration of the lethal concentration, and 3) the rate of translocation of these insecticides in beet seedlings. This paper reports the results of these experiments and points out how this information may explain virus spread under field conditions.

MATERIALS AND METHODS.—The experiments were carried out during 1955 with yellows virus from local infected table beet plants. Experiments were repeated during 1956 with yellows virus obtained from C. W. Bennett, Sugar Crops Section, U. S. Department of Agriculture, Salinas, California. The latter virus causes a distinct initial vein clearing not noted as a primary symptom in the Puget Sound region; however, both the ultimate symptoms in beets and the aphid vector relations are similar regardless of virus source.

Yellows virus was obtained from caged, mature sugar beet plants that showed marked yellows symptoms during the course of these studies. Green peach aphids were transferred from healthy spinach seedlings to the yellows-affected beets and permitted to colonize. Apterous aphids that had fed for 24 hours or longer on infected beets were used in most transmission studies.

Seedlings of the sugar beet variety US 75, maintained in 2-in. pots and grown under aphid-free conditions, were used as test plants in all experiments. This variety is vigorous and has leaves on which primary symptoms are readily recognized. Thirty-five seedlings, in the 4- to 8-leaf stage, were carefully sprayed with each of the following emulsifiable insecticides diluted according to the manufacturer's recommendations: Thimet (47.5 per cent *O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate), 1.25 ml/1; 26.2 per cent demeton (*O,O*-diethyl *S*(and *O*)-2-(ethylthio)ethyl phosphorothioates), 1.25 ml/1; 25 per cent parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate), 2.5 ml/1; Metacide (10 per cent parathion plus 40 per cent *O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate), 1.25 ml/1; and 57 per cent malathion (*S*-[1,2-bis (ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorodithioate), 1.87 ml/1.

Twenty-five plants from each treatment were arranged in groups of 5. After the sprays had dried (approximately ½ hour), 10 aphids were transferred by means of a moistened camel's-hair brush from infected beets to each of 5 cages (3), each of which was clamped onto a leaf of an insecticide-treated plant of each group. After 20-minute test feeding periods (including a 5-minute prepenetration time and a 15-minute feeding interval), each group of aphids was transferred consecutively to each of the other 4 plants of the group. The aphids fed 20 minutes on each plant. Following the last transfer (after 100 minutes), the aphids were inactive and unable to feed, regardless of the insecticide used. Watson (6,7) showed that a 15-minute test feeding is suitable for transmission of the yellows virus after a 24-hour acquisition feeding. The inoculated plants in each group from each treatment were fumigated with Nicofume and transferred to bench soil for observation of yellows symptoms. Symptoms were pronounced in inoculated plants after 20 days.

Similar lots of aphids were caged on each of the remaining plants from each insecticide treatment and

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TABLE 1.—*Transmission of beet yellows virus by groups of 10 viruliferous green peach aphids in 5 serial transfers for 20-minute feeding periods on insecticide-treated plants of US 75 sugar beet, as indicated by presence of yellows symptoms in 20 days, Mount Vernon, Washington, 1956*

Aphid group	Presence (+) or absence (—) of symptoms in plants treated with indicated insecticide and then fed upon by viruliferous aphids during 5 consecutive 20-minute test feedings																								
	Thimet					Demeton					Parathion					Metacide					Malathion				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
A	+	+	+	+	+	+	—	—	—	+	+	+	—	+	—	+	—	—	—	—	—	+	+	+	—
B	—	—	—	—	—	+	—	+	—	+	—	—	—	—	+	—	+	+	+	+	—	—	+	—	—
C	—	—	—	—	—	+	+	+	—	+	+	+	+	+	+	—	+	—	—	—	—	—	—	—	+
D	—	—	—	—	—	+	—	+	—	—	—	—	+	+	—	—	—	—	—	—	—	+	+	—	—
E	—	—	+	—	—	+	+	—	+	—	—	+	—	—	—	—	—	—	—	—	+	+	—	—	—

permitted 24-hour consecutive feeding periods. Dead aphid colonies were removed at each 24-hour interval, and a new set of 10 aphids was placed in the cages. The experiment was continued until most of the aphids remained alive and feeding after the 24-hour period. Toxic quantities of an insecticide were considered to be dissipated when aphids were alive after a 24-hour feeding period on treated plants.

In another experiment, each insecticide was sprayed on the foliage of each of 10 beet plants, except for a single unsprayed leaf protected by enclosure in an inverted test tube stoppered with cotton. Ten aphids were caged on each unsprayed leaf for 8-hour feeding periods. Aphids were replaced at the end of each feeding period, and the experiment was continued until it could be determined whether the insecticide was translocated to the unsprayed leaf in lethal amounts.

RESULTS.—Viruliferous aphids permitted 20-minute test feeding periods in successive transfers to insecticide-treated plants were not inactivated until they had fed 80–100 minutes. The time necessary to inactivate aphids fell within the same range for each insecticide tested. Some aphids maintained ability to transmit the yellows virus to insecticide-treated plants until they were killed (Table 1).

Duration of the lethal residual and/or systemic action of the different insecticides was determined; there does not seem to be a sharp period of dissipation for each insecticide. Thimet and demeton killed feeding aphids for 18–24 days after application in separate tests. Only Metacide was slightly phytotoxic to sugar beets, but it had greater residual action (12–16 days) than did parathion (8–14 days) and malathion (6–10 days).

Sugar beet seed were coated with Thimet 44-D (44 per cent Thimet and 2 per cent methyl cellulose in carbon powder) by agitating seed with the powder and then planted in greenhouse bench soil. Aphids caged on seedling leaves 21 days old (large enough to hold cages) were not killed after continuous feeding. Evidently, Thimet was not effective in leaves 21 days after application as a seed treatment.

In several tests, Thimet and demeton were translocated from sprayed to unsprayed leaves within 18–24 hours after application. The lethal duration of translocated materials was as long as that in sprayed leaves. Lethal amounts of parathion, Metacide, and malathion were not translocated.

DISCUSSION.—Conditions of the experiments were not completely analogous to those in the field. Nevertheless the results might help explain why effective field control of virus yellows has not resulted from use of insecticides for aphid control. Watson (6) demonstrated that the yellows virus is persistent in the green peach aphid. Viruliferous aphids may be carried great distances and over long periods and retain their ability to transmit the virus. Viruliferous alate aphids migrating into beet fields could inoculate a number of insecticide-treated plants before being killed by the insecticide. After short feeding periods on insecticide-treated plants, aphids become irritable and might fly away to feed on other plants rather than to remain on a single plant.

In another experiment, migratory alate aphids were trapped in water-filled yellow cake pans placed in steckling plant beds. Aphids were removed periodically and counted. Populations included a number of undetermined species, but the green peach aphid predominated in several locations. The origin of the trapped aphids was not known, and it was not determined whether they were viruliferous; however, populations of trapped green peach aphids were so large that if only a very small percentage were viruliferous they could make sufficient inoculations during the course of a season to establish the disease in serious proportions.

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EFFECT OF CROP, CROP RESIDUES, TEMPERATURE, AND MOISTURE ON SOIL FUNGI[1]

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SUMMARY

Some of the factors that may be operative in inducing changes in mycofloras in the field were investigated in the laboratory and greenhouse. Mycofloras of soil were different after 4 successive croppings of alfalfa, corn, oats, or wheat. Highest numbers of colonies of *Penicillium funiculosum* series and *P. purpurogenum* series were isolated from corn soil; *Fusarium* colonies were highest in alfalfa soil and lowest in corn soil. Highest numbers of colonies of *Rhizopus* spp. were associated with wheat.

The mycofloras of soil cropped to corn and oats were different from soils amended with the residues of these crops.

Soil moistened daily to field capacity and maintained for 5 weeks at 10°, 20°, or 30°C did not differ in the qualitative or quantitative nature of the mycofloras.

After 7 weeks, there were differences in the mycofloras of soils watered at different time intervals, with consequent moisture content fluctuations from 20 to 8 per cent, from 20 to 14 per cent, and from 20 to 18 per cent by weight.

Apparently the pH of soils was not a factor contributing to mycofloral changes in this investigation.

It appears that the crop plant is a decisive factor influencing soil mycofloras. The direct influence of seasonal changes in soil temperature, moisture, and pH on mycofloras may not be appreciable. The mycofloras associated with different crops appear to be characteristic; therefore, the use of crop sequences is an effective and practical method of altering soil mycofloras.

A certain degree of control of root rots of field crops has been achieved by suitable cultural practices such as crop sequences or soil amendments. This reduction in severity of root diseases has in many cases been attributed to changes in the nature and activity of the soil mycofloras in relation to various soil-borne pathogens. Total numbers of microorganisms in the soil have been correlated with the effects of cropping sequences and other cultural practices on disease incidence. Deems and Young (6) recently demonstrated that the control of a specific pathogen, *Aphanomyces cochlioides* Drechs., could be correlated with prevalence of specific fungi, or groups of fungi, rather than with total numbers. The demonstration that mycofloral changes are induced by various crops and cropping sequences and a knowledge of the specific factors influencing these changes in mycofloras are of fundamental importance to an understanding of the relationship of crop sequences to root rot incidence. Some of the factors that may be operative in changing the mycofloras under different crops in the field were investigated under controlled conditions in the laboratory and greenhouse. The factors under consideration were 1) cropping sequences and soil amendments, 2) soil temperature, and 3) soil moisture.

Materials and methods.—All soils studied were a mixture of Wooster silt loam top soils. Soil samples were assayed for fungi by removing 8 cores from each treatment replicate by inserting a ½-in. cork borer perpendicular to the surface to a depth of 2 in. The soil from each replication was then sieved, pooled, and mixed thoroughly before it was assayed for fungi. A soil-dilution plate technique, as modified at the Ohio Agricultural Experiment Station, was used for the assay of fungi.[3] A 250-mg subsample was removed from each thoroughly pooled sample, added to 50 ml of 1 per cent cellulose gum (CMC[4]), and stirred with a mechanical stirrer for 1 minute. One dilution of this suspension was made; this resulted in a final dilution of 1:10,000. One ml of the final suspension was

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[3] Schmitthenner, A. F. Unpublished data.
[4] CMC = 120 high viscosity carboxy-methyl cellulose furnished by Hercules Powder Company, Wilmington, Delaware.

pipetted into petri plates and mixed with 10 ml of warm Ohio Agricultural Experiment Station (OAES) medium, the basal ingredients of which were 20 g purified agar, 5 g glucose, 2 g yeast extract, 1 g sodium nitrate, 0.5 g magnesium sulfate, 1 g potassium phosphate (monobasic), 50 mg streptomycin sulfate, 50 mg Chloromycetin (chloramphenicol), 1 g oxbile, and 1 g sodium propionate per liter.

After preparation, the soil plates were incubated for 5 days at 24°C; then each colony was numbered on the bottom of the plate. Colonies appearing after the incubation period were considered to be secondary. There is a possibility that some slow-growing fungi might be overlooked in this procedure. The fungi were identified to genus, except in the case of the Aspergilli and the Penicillia, which were identified to the groups or series as described by Thom and Raper (18) in the *Manual of Aspergilli* and by Raper and Thom (15) in the *Manual of Penicillia*. Several other publications also were used for the purpose of identification (1, 2, 3, 4, 10). Total numbers of fungi were calculated on the basis of oven-dried soil.

The analysis of variance test was applied to determine the differences between the occurrence of the various groups of fungi. According to some investigators (12, 13), the distribution of fungus colonies on soil-dilution plates follows a Poisson distribution. With this type of distribution, the variance becomes proportional to the mean; therefore, it is necessary to make appropriate transformations to divorce the mean and variance in order to make the analysis of variance test valid. According to Freeman and Tukey (8) appropriate square root transformations are most suited for this purpose. The resulting changes in numerical values do not impair the experimental significance.

EXPERIMENTAL RESULTS.—*Effect of continuous cropping on soil mycofloras.*—To determine whether different and perhaps characteristic mycofloras would develop in soils continuously cropped to alfalfa, corn, oats, or wheat, soil was collected from an alfalfa field, thoroughly mixed, sieved, and divided into 2 equal portions. One half of the soil was autoclaved for 4 hours in order to facilitate a later study of fungus recolonization of sterile and nonsterile soils. The autoclaved and nonautoclaved soils were then placed in $2 \times 2 \times 1$-ft. wooden boxes. Eight boxes of each soil were used. Two boxes of each soil were planted to alfalfa, corn, oats, or wheat and kept in the greenhouse. Three months later, the plants were removed, dried, chopped into small pieces, and incorporated with the soil. The croppings and the incorporation of plant residues with the soil were repeated 4 times during a 2-year period. At the end of the fourth planting, soil samples were collected from each box and assayed for fungi.

Different mycofloras developed in these continuously cropped soils (Table 1). Alfalfa was distinctly different from the others in having the highest number of colonies of total Aspergilli and members of the following: *Aspergillus ochraceus* group, *A. ustus* group, *Fusarium* spp., and *Penicillium rugulosum* series. Corn soil contained the highest number of colonies of *P. funiculosum* series, *P. purpurogenum* series, and total Penicillia. Oat soil had the highest number of colonies of *Chaetomium* spp. Wheat soil was characterized by the highest number of colonies of *Rhizopus* spp. There appeared to be an association between the occurrence of colonies of *Fusarium* spp. and *P. funiculosum* series; high numbers of one associated with low numbers of the other. Such an inverse relationship between the 2 fungus groups was most striking in the case of alfalfa and corn soils. Colonies of *P. funiculosum* series occurred in greatest numbers in corn soil, whereas the number of colonies of *Fusarium* spp. was relatively low. In alfalfa soil, the opposite pattern existed; very high numbers of colonies of *Fusarium* spp. and low numbers of colonies of *P. funiculosum* series were isolated from it.

TABLE 1.—*Frequency of occurrence of fungi in greenhouse soils continuously cropped to alfalfa, corn, oats, or wheat for 2 years*

Fungi[a]	Transformed mean[b] of number of colonies from indicated soils				LSD 0.05
	Alfalfa	Corn	Oats	Wheat	
Aspergillus fumigatus group	1.7	2.6	1.8	1.7	0.6
A. ochraceus group	2.2	0.6	0.6	0.6	0.1
A. ustus group	2.7	1.3	1.5	1.6	0.5
Total Aspergilli	3.1	1.3	0.9	1.1	0.4
Chaetomium spp.	0.7	1.1	2.6	1.2	0.2
Fusarium spp.	2.1	0.9	1.0	1.1	0.3
Penicillium funiculosum series	0.8	2.3	1.1	1.0	0.4
P. purpurogenum series	0.6	1.5	0.7	1.3	0.3
P. rugulosum series	3.4	2.9	3.0	2.4	0.4
Total Penicillia	3.9	4.5	3.3	3.1	0.4
Rhizopus spp.	1.9	1.5	0.3	2.1	0.3
Trichoderma spp.	1.5	2.0	2.0	1.7	
Totals of all fungi	6.4	5.6	5.1	4.8	0.3

[a] Fungi occurring in greatest numbers were selected for statistical analysis. An LSD is given where differences between treatments were statistically significant.

[b] Mean number of colonies derived from 4 replicates of 8 petri plates. The square root transformations recommended by Freeman and Tukey (8) were used to transform individual plate data.

Effect of crops as compared with their incorporated residues on soil mycofloras.—The differential effect of crops as compared with their incorporated residues was also investigated. Equal quantities of soils were collected from corn and oat fields; they were sieved, mixed thoroughly, and then placed in 1-gal. glazed clay crocks. Three grains of corn or 6 of oats were planted in each crock containing 8 lb. of soil. In 1 series of crocks, finely ground residues of corn or oat plants were mixed individually with soil at the rate of 7 g for every 8 lb. of soil. On a field basis, this is equivalent to about 2 tons of plant residue per acre, which is approximately the amount of dry plant material plowed down after each of the respective crops. The soil-residue mixtures were then placed in crocks.

Soil in crocks without plants or plant residues were maintained as checks or fallow. Treatments were replicated 4 times. The crocks were kept on greenhouse benches and watered regularly. After 2½ months, soil samples were taken from each crock and assayed for fungi.

Growing oat and corn plants influenced the relative frequency of several groups of fungi differently (Table 2). When compared to the fallow treatment, corn was the most distinct in mycoflora, whereas the corn-residue treatment was most similar. The oat and oat-residue treatments were similar in the number of fungus groups, differing in frequency from those of the fallow, and consequently were relatively similar to each other. Corn soil was distinct in having significantly higher numbers of colonies of *P. funiculosum* series, of total Phycomycetes, and of *Rhizopus* spp. than did oat soil or soil amended with corn or oat residue. Higher numbers of colonies of *Fusarium* spp., *Gliocladium* spp., *P. decumbens* series, and *P. regulosum* series were isolated from oat soil than from corn soil. The least number of colonies of *P. funiculosum* series was isolated from oat soil and from soil amended with oat straw. It also may be observed in Table 2 that the differences between the mycofloras of soils amended with corn and of those amended with oat residue were not as striking as those between soils cropped to corn and oats. Significantly higher numbers of colonies of *Fusarium* spp., *P. decumbens* series, *P. funiculosum* series, and total numbers of fungi occurred in soil amended with corn residue than in that amended with oat residue; in contrast, *Trichoderma* spp., *Rhizopus* spp., and *P. rugulosum* series occurred more frequently in soil amended with oat residue than in soil amended with corn residue. The numbers of colonies of *Fusarium* spp., *Gliocladium* spp., *P. decumbens* series, and *P. funiculosum* series in soil amended with oat residue were less than in the fallow (check). That the incorporation of oat residue with soil reduces incidence and severity of root-rot organisms, such as *Fusarium* spp., has been reported previously (13, 21). Certain similarities may be observed between the results of this experiment and those of the investigations concerning the effect of 2-year continuous cropping of alfalfa, corn, oats, or wheat on soil mycofloras (Table 1). The most striking similarity is the predominance of colonies of *P. funiculosum* series in corn soil. Similarly, the relationship of *P. funiculosum* series and *Fusarium* spp. is also of interest. When the frequency of occurrence of colonies of *P. funiculosum* series was high, the numbers of colonies of *Fusarium* spp. was low.

TABLE 2.—*Frequency of occurrence of fungi in soils cropped to corn or oats, as compared with that in soils amended with corn or oat residues or with fallow (check) soil*

Fungi[a]	Transformed mean[b] of number of colonies from indicated soils					
	Corn	Oat	Corn residue	Oat residue	Fallow (check)	LSD 0.05
Fusarium spp.	1.0	1.6	1.5	1.2	1.5	0.3
Gliocladium spp.	1.2	1.7	1.9	1.5	2.2	0.5
Mucor spp.	1.3	1.4	1.7	1.4	1.5	
Penicillium decumbens series	1.0	1.3	1.3	1.1	1.4	0.2
P. funiculosum series	2.0	0.8	1.1	0.7	1.1	0.3
P. restrictum series	1.4	2.1	1.4	1.7	1.5	0.4
P. rugulosum series	1.3	1.8	1.7	1.9	1.5	0.2
Total Penicillia	2.7	2.6	2.7	2.5	2.4	
Total Phycomycetes	1.8	1.0	1.1	1.1	1.0	0.2
Rhizopus spp.	3.1	1.3	1.0	1.4	1.0	0.4
Trichoderma spp.	1.5	1.6	1.3	1.5	1.3	0.2
Totals of all fungi	4.2	4.5	4.6	4.3	4.2	0.3

[a] Fungi occurring in greatest numbers were selected for statistical analysis. An LSD is given where differences between treatments were statistically significant.

[b] Mean number of colonies derived from 4 replicates of 8 petri plates. The square root transformations recommended by Freeman and Tukey (8) were used to transform individual plate data.

Fungus recolonization of autoclaved soil.—The recolonization of autoclaved soil by fungi as compared with the mycofloras of the same but nonautoclaved soil was investigated in conjunction with the study of the effects of continuous cropping on soil fungi. Portions of autoclaved and of nonautoclaved alfalfa field soil were placed in boxes in the same location in the greenhouse and continuously cropped to alfalfa, corn, oats, or wheat. At the end of the 2-year test period, the predominant fungi in both the autoclaved and nonautoclaved soils were approximately the same (Table 3). A total of 23 genera, including 11 groups of Aspergilli and 8 series of Penicillia were identified. Only 7 genera, groups, or series were not isolated from both autoclaved and nonautoclaved soils; of these, 5 types were isolated from 1 or more nonautoclaved soils and 2 from 1 or more autoclaved soils. All of these types that did not appear in both autoclaved and nonautoclaved soils were extremely low in occurrence.

Chaetomium spp. and *Rhizopus* spp. were distinct in that the highest number of colonies of these fungi occurred in autoclaved soil in 3 of 4 treatments. It is interesting to note from the results of this experiment that recolonization of autoclaved soil by the same predominant groups of fungi as those present in similarly cropped nonautoclaved soil was achieved in a relatively short time.

Effect of temperature and moisture on soil mycofloras.—Since environmental factors may induce changes in mycofloras in the field, the effects of soil temperature and of moisture on the occurrence of various groups of fungi were studied. Soil from corn and oat fields was mixed and air dried; necessary precautions to avoid contamination by air-borne microorganisms in the laboratory were taken. The moisture content of this soil at field capacity, determined according to the procedure outlined by Meyer and Anderson (14), was approximately 20 per cent by weight.

To determine the effect of moisture on soil mycofloras, 150-g samples of air-dried soil were placed in each of 12 plastic cups. Water was added to each cup to bring the soil moisture content near the field capac-

TABLE 3.—*Frequency of occurrence of fungi isolated from autoclaved and nonautoclaved soils after being continuously cropped to alfalfa, corn, oats, or wheat for 2 years*

Fungi[a]	Transformed mean[b] of number of colonies from autoclaved (A) and nonautoclaved soil (NA)								LSD
	Alfalfa		Corn		Oats		Wheat		
	A	NA	A	NA	A	NA	A	NA	0.05
Aspergillus fumigatus group	1.3	2.2	2.6	2.6	2.1	1.5	1.9	1.5	0.8
A. ochraceous group	1.8	2.7	0.6	0.7	0.7	0.6	0.6	0.7	0.2
A. ustus group	2.3	3.1	1.0	1.6	1.4	1.5	1.0	2.1	
Total Aspergilli	2.4	3.8	1.1	1.5	1.1	0.8	0.8	1.7	0.6
Chaetomium spp.	0.7	0.7	1.3	0.9	3.2	2.0	1.7	0.7	0.3
Fusarium spp.	2.4	1.8	0.9	0.9	0.9	1.0	0.8	1.3	0.4
Penicillium funiculosum series	0.7	0.9	1.9	2.7	1.2	0.9	1.1	0.9	0.6
P. purpurogenum series	0.6	0.6	2.3	0.7	0.7	0.6	0.7	1.7	0.4
P. rugulosum series	3.6	3.1	3.1	2.7	3.1	2.8	2.4	2.4	
Total Penicillia	3.7	4.0	4.8	4.2	3.5	3.0	2.7	3.4	0.5
Rhizopus spp.	2.5	1.2	1.4	1.6	0.5	0.0	2.5	1.6	0.5
Trichoderma spp.	1.5	1.5	2.6	1.4	2.6	1.4	1.1	2.2	0.8
Totals of all fungi	6.3	6.6	5.8	5.3	5.6	4.7	4.7	4.9	0.4

[a] Fungi occurring in greatest numbers were selected for statistical analysis. An LSD is given (for autoclaved versus nonautoclaved soils only) where differences between treatments were statistically significant.

[b] Mean number of colonines derived from 2 replicates of 8 petri plates. The square root transformations recommended by Freeman and Tukey (8) were used to transform individual plate data.

ity, then the weight of each cup plus soil and water was recorded. The weighed cups of soil were placed in a room maintained at 25°C and constant relative humidity. The cups were covered with a double layer of cheesecloth to reduce the fall of air-borne spores on the soil surface. Soil moisture was then controlled by the addition of water at different time intervals. The moisture content of the soil fluctuated from 20 to 8 per cent in soils watered every 6 days, from 20 to 14 per cent in soils watered every 4 days, and from 20 to 18 per cent by weight in soils watered daily. At the end of 7 weeks, the soil from each cup was removed, mixed, and assayed for fungi.

A list of the predominant groups of fungi and their frequency of occurrence in soils maintained at these 3 different moisture levels is presented in Table 4. The predominant groups of fungi isolated from soils with different moisture contents were the same, but there were differences in the relative frequency of their occurrence. The number of colonies of *Botrytis* spp., of *P. restrictum* series, and of total Penicillia decreased as moisture content of the soil was increased. Within limits of this experiment, the total numbers of fungi were significantly less at higher moisture contents than at lower ones.

To study the effect of soil temperature on the frequency of occurrence of fungi, quadruplicate soil samples were stored at 10°, 20°, and 30°C in plastic cups. The moisture content of the soil was maintained at field capacity (20 per cent by weight) by daily addition of appropriate quantities of water. At the end of 5 weeks, the soil from each cup was mixed and assayed for fungi.

The predominant fungi in soils maintained at these different temperatures were the same. Statistically significant differences between treatments in the frequency of occurrence of various groups of fungi or total numbers of fungi were not observed. It was surprising that significant differences were not observed in the mycofloras of soils maintained at the 2 relatively extreme temperatures. In Ohio, during the growing season, soil temperatures rarely attain either of the extreme temperatures of this experiment, i.e. 30°C (86°F) or 10°C (50°F).

It is apparent that soil temperature and moisture do not influence the mycofloras as greatly as do the growing plants.

DISCUSSION.—The soil-dilution plate method in its diverse forms has been widely used by many investigators in assaying soils for their qualitative and quantitative microbial content. The dilution plate method is most useful in quantitative microbial studies,

TABLE 4.—*Frequency of occurrence of fungi in soils maintained at different moisture levels for 7 weeks*

Fungi[a]	Transformed mean[b] of number of colonies from soils with indicated moisture contents (in per cent)			LSD
	20-8	20-14	20-18	0.05
Botrytis spp.	1.7	1.3	1.3	0.3
Fusarium spp.	1.1	1.3	1.1	
Gliocladium spp.	1.3	1.2	1.0	
Mucor spp.	1.1	1.3	1.8	
Penicillium funiculosum series	1.3	1.2	1.2	
P. restrictum series	1.9	1.7	1.2	0.3
Total Penicillia	2.5	2.3	2.0	0.3
Total Phycomycetes	1.3	1.4	1.5	
Trichoderma spp.	1.8	1.6	2.0	
Totals of all fungi	3.7	3.5	3.3	0.2

[a] Fungi occurring in greatest numbers were selected for statistical analysis. An LSD is given where differences between treatments were statistically significant.

[b] Mean number of colonies derived from 4 replicates of 8 petri plates. The square root transformations recommended by Freeman and Tukey (8) were used to transform individual plate data.

and perhaps it is the best method available for large-scale studies. How closely the true quantitative population of microorganisms is estimated by the use of the soil-dilution plate method is very debatable, but its use in comparing the occurrence of specific microorganisms between treatments should be valid.

The choice of a plating medium for studying the occurrence of soil fungi is difficult. The OAES medium was selected as the soil fungal assay medium for its convenience in the identification of colonies directly on the isolation plates. The medium is transparent, it eliminates bacteria and actinomycetes, and it restricts the growth of rapidly spreading fungi but at the same time supports the growth and sporulation of a wide range of fungi.

There is always the possibility of exclusion of a fungus or group of fungi by the adoption of a single plating medium. All fungi commonly isolated from soils by other soil plating media have been isolated with this medium. To date, over 100 genera have been isolated from soil by the use of the OAES medium. The majority of this list has been published (23). The support of growth of this wide range of fungi by this medium is considered adequate for the comparison of gross mycofloral changes in soils, and any selectivity this medium exerts on mycoflora determinations should be exerted equally on all treatments.

The establishment of different mycofloras under different crops has been demonstrated by some investigators. Herr (12) found that different mycofloras developed in soils continuously cropped to corn, oats, or wheat for 39 years. That the establishment of different mycofloras under different crops in the field may take place during 1 season was recently reported by Deems (5). The data from the present investigation leave little doubt that in the greenhouse different mycofloras develop in 2–3 months in soils cropped to corn or oats. The differences in the mycofloral population were distinct when soils were continuously cropped to alfalfa, corn, oats, or wheat for 2 years. The mycofloras of the different crops appeared to become stabilized during the 2-year period. The differences in the kinds of fungi associated with the different crops are due primarily to the relative frequency of isolation and not to their complete absence in any treatment.

The differences in the mycofloras of soils amended with corn and oat residue were not as great as in soils cropped to corn and oats. Addition of oat straw to soil resulted in the reduction of colonies of *Fusarium* spp., a finding previously reported by Kommedahl and Brock (13). With the exception of *Fusarium* spp. and *P. restrictum* series, most of the fungi were equally abundant in soil cropped to oats and in soil amended with oat residue. In contrast, the relative frequency of most groups of fungi differed in soil cropped to corn and in that amended with corn residue. These results do not conform with the findings of Tyner (21), who investigated the effect of amendment of soil with wheat, oat, or barley straws. He found the bacterial and fungal populations highest in straw-amended soil. This high microbial count can be attributed to the excessive quantities of straw he added to the soil (3 per cent by weight or approximately 27 tons per acre), a situation that is abnormal under ordinary cereal crop rotations.

The effect of environmental factors such as soil temperature, moisture, or pH on soil mycofloras may have been overemphasized in the past. Fluctuations have been reported in total numbers of bacteria, fungi, or actinomycetes due to changes in soil temperature and moisture (7, 11, 16, 17, 22). In this investigation, there were no qualitative or quantitative differences in mycofloras of soils maintained for 5 weeks at 10°, 20°, or 30°C. Most fungi were equally abundant in soils with different moisture contents. Total numbers of fungi were greater at low than at high moisture contents; this difference was primarily due to smaller numbers of colonies of *Botrytis* spp., *P. restrictum* series, and total Penicillia.

Several questions arise from the studies of the effects of soil temperature and moisture on soil fungi. Will the same pattern in the occurrence of mycofloras prevail if the soils are maintained at different temperatures and moisture levels for long periods of time? If plants had been growing in soils kept under different environmental conditions, could one expect the effect of temperature and moisture on soil fungi to be the same as those without plants? The answers to these and other questions may not be obtained on the basis of the results of 2 short-term experiments reported here. These experiments were designed to determine the effects of environmental conditions during short periods. That variations in mycofloras do take place in short periods has been demonstrated by Deems (5), who isolated fungi from differently cropped soils at weekly intervals. Also, differences in mycofloras were detected in about 3 weeks by Timonin (19, 20) in soils treated with root diffusates of 2 varieties of flax and of tobacco. It is therefore logical to assume that the 5- to 7-week test period in this investigation was sufficient to detect differences in the mycofloras of soils maintained at the different temperatures and moisture levels. The direct influence of seasonal changes in soil temperature and moisture on mycofloras may not be very appreciable. Of far more importance is the indirect effect, the influence of season changes on the crop plants that in turn supply the substrates used by the fungi.

Apparently, the pH of soils was not a factor contributing to mycofloral changes in this investigation. Soil pH was determined at the conclusion of each experiment and even in situations where the mycofloras were different, the pH values were the same.

It may be concluded from this investigation that the crop plant is a decisive factor influencing the mycofloras of soils. This effect may be brought about as a result of chemical substances diffusing into the soil from the roots of plants and the sloughing off of materials from roots; these may promote or retard the growth and activity of various groups of fungi. As Garrett (9) pointed out, the distribution of fungi is de-

pendent on the nature and amount of utilizable substrates in the soil. Since the crops and crop residues were found to influence the mycofloras differently, the amendment of soil with crop residues cannot in all cases be a substitute for the growing crops to obtain the same soil mycofloras. The mycofloras associated with different crops appear to be characteristic. As rotations are commonly practiced in agriculture today, the use of proper crop sequences is not only an effective but also a practical method of altering soil mycofloras.

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MISSISSIPPI CROWDER, A NEW DISEASE-RESISTANT COWPEA[1]

W. W. Hare

SUMMARY

Mississippi Crowder, a new variety of cowpea, is a midseason, large-vined variety very similar in type to the Brown Sugar Crowder. Mississippi Crowder is highly resistant to Race 1 and tolerant to Races 2 and 3 of *Fusarium oxysporum* f. *tracheiphilum*.

The brown crowder type of cowpea has been one of the leaders among the types of varieties used as a vegetable. Brown Sugar Crowder has been the most prominent variety of this type and popular for fresh-market, freezing, and canning. All brown crowders tested have been susceptible to Races 1, 2, and 3 of *Fusarium oxysporum* f. *tracheiphilum* (E.F. Sm.) Snyd. & Hans.

ORIGIN OF MISSISSIPPI CROWDER.—Adjacent to a wilt-variety test in 1949, about ½ acre of Brown Sugar Crowder was planted in soil infested with Race 1 of the wilt organism. There were several plants that survived, but only 1 resembled Brown Sugar Crowder. Progenies from this plant were selfed and tested for wilt resistance in the greenhouse for 5 generations. There were no recognizable variations in type except for 1 of 117 lines in the first generation. Wilt resistance remained the same after the third generation. The highest yielding lines of the fifth generation were bulked and increased, and foundation seed was grown in 1955. Seed was released to seed producers for the 1956 season as the variety Mississippi Crowder. Adequate seed for all purposes was available for the 1957 growing season.

DESCRIPTION OF MISSISSIPPI CROWDER.—Mississippi Crowder is very similar to Brown Sugar Crowder in type, although in close comparison there are many minor differences. It is midseason in maturity, with large spreading vines and large dark-green leaves. Pods are borne scattered at the foliage level. They are dark green through the fresh-market stage, turn yellow briefly, and dry to a straw color. Average sizes are 6½ in. long and 0.38 × 0.44 in. in cross section (Fig. 1,D). The peas are "crowded" in the pod and flattened on the ends. They are a light cream color at fresh-market stage and light tan when dry.

DISEASE RESISTANCE.—In field plots, Mississippi Crowder was tolerant to virus diseases and was not as severely damaged by the curculio and weevil as were many other edible varieties. These are also characteristics of Brown Sugar Crowder. In greenhouse tests in soil infested with 4 species of root-knot nematodes, average root-knot indices were 62.5 for Brown Sugar Crowder and 46.7 for Mississippi Crowder. Brown Sugar Crowder was susceptible to all races of the Fusarium wilt organism. Mississippi Crowder was highly resistant to Race 1 and tolerant to Races 2 and 3 (Fig. 1, A, B, C).[2] There were wide differences in the incidence of wilt in replicated field plots infested with all 3 races; e.g. in 1954, an average of 81.5 and 12.8 per cent of the plants of Brown Sugar Crowder and Mississippi Crowder, respectively, were killed by wilt.

TABLE 1.—*Summary of yields of Mississippi Crowder compared to Brown Sugar Crowder in pounds of green pods per acre*

Variety	Year 1953	1954	1955	1956	Average	Relative yield
(On Fusarium-infested plots at State College, Miss.)						
Brown Sugar Crowder	4074	2884	2775		3244	100.0
Mississippi Crowder	7170	5222	4412		5601	172.7
(Southern variety trials*—Fusarium infestation unknown)						
Brown Sugar Crowder	3723	2511	2691	2460	2846	100.0
Mississippi Crowder	4066	3110	3699	2946	3455	121.4

* Cooperative trials in the southern region; tested at 10 locations in 6 states in 1953, 9 locations in 4 states in 1954, 10 locations in 5 states in 1955, and 13 locations in 7 states in 1956.

COMPARATIVE YIELDS.—In paired plots with only light damage from wilt late in the season, there was no significant difference between the yields of Brown Sugar Crowder and Mississippi Crowder. It appeared that there was no intrinsic difference in yielding ability of the 2 varieties. Differences in yields elsewhere could probably be attributed primarily to the greater inherent resistance or tolerance to Fusarium wilt.

Over a 3-year period, Mississippi Crowder produced 72 per cent more yield than did Brown Sugar Crowder when grown in replicated field plots known to be infested with all 3 races of *F. oxysporum* f. *tracheiphilum* (Table 1). Relative proportions of the 3 races were not determined, but all 3 were recovered from wilted plants in the plots. In 4 years in the Southern Variety Trials (under the label Mississippi S-1 in 1953 and 1954), the average yield of Mississippi Crowder was 21 per cent greater than that of Brown Sugar Crowder. Compared to all types tested in the trials, the yield rank of Mississippi Crowder was fifth in 1953, third in 1954, first in 1955, and third in 1956. In these trials, Mississippi Crowder was not surpassed

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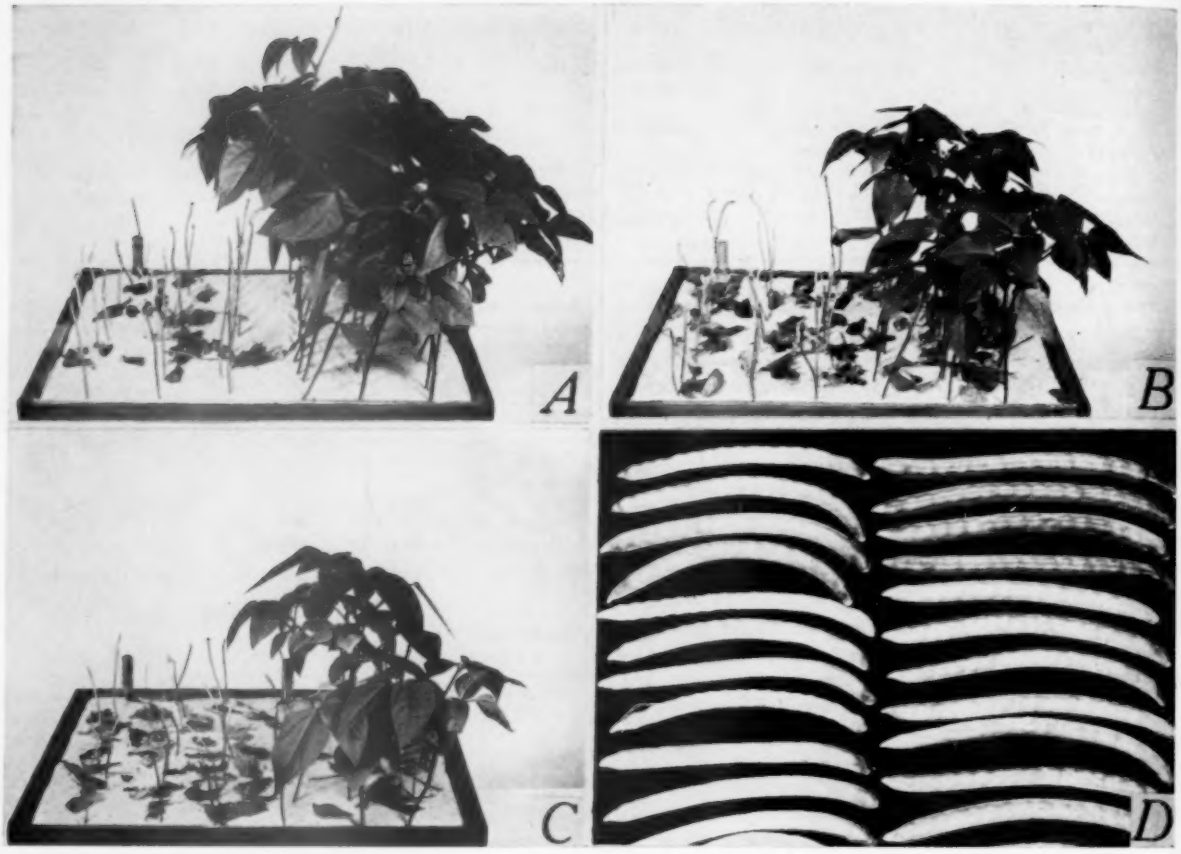

FIG. 1. Left, Brown Sugar Crowder; right, Mississippi Crowder. A) Inoculated with Race 1 of *Fusarium oxysporum* f. *tracheiphilum*. B) Inoculated with Race 2. C) Inoculated with Race 3. D) Mature pods of both varieties.

in ranking for uniformity, appearance, and pod-fill. It was rated in the medium group for percentage shell-out and ease of shelling.

Large-scale trials were conducted from 1954 through 1956 in cooperation with commercial canners. Yield and canning quality were reported as satisfactory to superior. Particular attention was directed to ease of shelling, and no difficulties in shelling were encountered when standard viners were used. One large-scale fresh-market test and 1 small one were made in 1956. High yields were obtained, and buyers accepted the product readily.

No commercial freezing tests of Mississippi Crowder have been made. The variety was included by Jenkins[3] in an investigation of freezing and nutritional qualities. He concluded that Mississippi Crowder was equal or superior to all varieties used.

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[3] Jenkins, W. F. 1957. Varietal desirability and nutritive value of southern peas before and after freezing and cooking. Proc. Amer. Soc. Hort. Sci. (In press.)

PHYTOTOXICITY OF CAPTAN AS INFLUENCED BY FORMULATION, ENVIRONMENT, AND PLANT FACTORS[1]

Robert H. Daines,[2] R. J. Lukens,[3] E. Brennan,[4] and I. A. Leone[4]

SUMMARY

Field and laboratory studies have indicated that the phytotoxicity of captan is increased under certain conditions. Factors involved in formulation, environment, and plant status were considered. Greenhouse tests showed that injury was increased as the concentrations of captan and wetting agent in the preparation were increased. The addition of kaolinite as a filler also increased phytotoxicity of the mixture. The presence of calcium carbonate and magnesium oxide had a "safening" effect. Captan toxicity to bean foliage increased with increasing temperatures and with decreasing light intensity. Laboratory studies *in vitro* have indicated that captan decomposition is accelerated by increasing temperature and time, with a resulting increase in hydrogen ion concentration, chloride concentration, and hydrogen sulfide accumulation. It is believed that this acidity may be partially responsible for captan phytotoxicity. It was also learned that amino compounds containing sulfhydryl groups cause rapid decomposition of this fungicide.

Captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2,-dicarboximide), developed by the senior author in cooperation with A. R. Kittleson, Chemist of the Standard Oil Company, has been proved fungicidal against many disease-producing organisms.[5, 6] In addition to combatting some common apple diseases, captan also has been effective in improving the finish of apple fruit; however, the foliage of Red Delicious, Paragon, and Baldwin apple varieties may develop small, circular, injured spots following the use of this fungicide. These spots most frequently develop during warm periods of low light intensity; although they usually appear near the blooming period, they may occur any time during the growing season. In 1953, captan injury to Red Delicious apples was particularly common in orchards in central New Jersey. Peaches sprayed with this fungicide frequently developed a leaf-lacing effect from the loss of injured tissue. When captan was first tested in the greenhouse against powdery mildew of bean, severe leaf scorch occasionally was encountered. Since the factors involved in this injury were unknown, a study of the effect of formulation, environment,[7] and plant factors on phytotoxicity was initiated.

MATERIALS AND METHODS.—*Phaseolus vulgaris* L. var. Bountiful, one of the species frequently exhibiting phytotoxicity, was used in most of the greenhouse studies. Plants were treated when the first trifoliate leaves had fully expanded, at which time the primary leaves were removed to facilitate the experimentation. The various treatments were applied with a medicine dropper to the lower leaf surface, the area most susceptible to injury. All materials were applied to each leaflet; 1 drop of each was applied to each of 48 leaves. In studies on the effect of temperature on injury, uniform plants were exposed for designated periods in thermostatically controlled chambers. Observations were made on the fifth day after treatment, at which time each treated spot was rated on amount of injury, and the mean percentage injury was reported as an average for all replications.

Spraying of trees was accomplished in the field with an 8-cluster nozzle gun at 500 lb. pressure; in the greenhouse, the sprays were applied in a spray tunnel by means of a 25-mph wind and water of varying temperatures.

The materials used in these tests were technical captan (97% pure); the diluents kaolinite, attapulgite, and neutral talc; and the wetting agent Igepal CA (isooctylphenol + ethylene oxide). All were supplied by the California Spray Chemical and Stauffer Chemical Companies. Surface tension measurements on all formulations were made with a du Nuoy tensiometer.

Similar suspensions of technical captan plus additives were employed in determining the accumulation of chloride and hydrogen ions, which were selected as criteria of captan decomposition. Two per cent suspensions of captan with varying ratios of the fillers were placed in a shaking machine in chambers kept constant at 50°, 75°, 90°, or 110°F. Samples of each suspension were tested for pH and for chloride ion content immediately and after 4 hours, 24 hours, and 1 week. The chloride content was determined by the Mohr titration method.[8] Resistance measurements also were made on the suspensions.

Drops of each suspension and of each filtrate were applied to bean leaves as described earlier and the extent of injury recorded. Drops of hydrochloric and sulfuric acids with pH values corresponding to those of the test materials also were applied to bean leaves and injury data taken.

RESULTS OF PHYTOTOXICITY STUDIES—*Formulation factors.*—1) *Concentration of captan.*—A dilution

[1] Accepted for publication April 27, 1957.
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[2] Plant Pathologist, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.
[3] Research Fellow.
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TABLE 1.—*Injury to bean leaves by various concentrations of captan formulated with 3 different diluents*

Captan concentration	Mean percentage injury[a] when diluent was as indicated		
	Kaolinite	Attapulgite	Talc
%			
0.25	2.31	6.00	0.00
0.50	21.30	15.24	0.65
1.00	26.73	33.69	2.22
2.00	41.00	—[b]	6.95
4.00	58.54	—[b]	19.26
8.00	85.46	—[b]	30.56

[a] Mean of 48 spots, each on a separate leaf; 1 drop of each treatment was applied to each leaf.
[b] Excessive flaking.

series of captan formulated with 3 different fillers, each in a 1:1 ratio, was tested to determine the influence of captan concentration *per se* on injury to bean foliage.

Injury increased with increasing concentrations of the fungicide in all formulations (Table 1). In this experiment, the results with different fillers cannot be compared, since the series were run separately under varying conditions of temperature and humidity.

2) *Fillers.*—Since captan is marketed in combination with a diluent and is usually used with other pesticides containing diluents, it was considered advisable to determine whether the diluents in common use alter the phytotoxic properties of the fungicide. Accordingly, 2 per cent mixtures of technical captan alone and in 1:1 formulations with kaolinite, attapulgite, and neutral talc were placed on the bean leaves as described earlier. All of the fillers used greatly increased injury over that produced by captan alone. Of the formulations used, the captan-kaolinite combination was the most phytotoxic, giving a mean percentage injury of 52.01 as opposed to 9.43 for captan alone. The captan-attapulgite and captan-talc combinations gave injury values intermediate between those of captan-kaolinite and captan alone. Injury was also increased as the proportion of filler to captan was increased (Table 2).

3) *Wetting agent.*—Since the amount of wetting agent in a captan mixture for field use may vary with the nature and quantity of ingredients and with the concentration of the spray mixture, experiments were conducted to determine the effect of wetting agents on captan injury. Suspensions containing Igepal CA, in amounts varying from 0 to 4 per cent of the weight of captan, were tested on bean leaves. An increase in the concentration of wetting agent resulted in a corresponding increase in plant injury (Table 3). High concentrations of wetting agent alone and in combination with kaolinite caused plant injury even in the absence of captan. Tensiometer measurements on these suspensions showed that the wetting agent caused a sharp decrease in surface tension (32 dynes/cm) up to 0.5 per cent concentration, beyond which there was no further decrease.

In an orchard experiment with Red Delicious apples, a similar but less pronounced effect occurred with talc. In this experiment, Igepal CA was added in varying amounts to a 1:1 captan-talc formulation (4 lb./100 gal.). Even with this different filler, the phytotoxicity of the mixture increased from 10 to 17.5 per cent as the concentration of the wetting agent was increased from 0.5 to 4.0 per cent.

Since the toxicity of captan increases with decreased surface tension of suspensions, stomatal penetration of leaves probably is involved. To test this possibility, Red Delicious apples trees mounted on a revolving platform were exposed in a spray tunnel to a suspension of technical captan (2 lb. per 100 gal.), at 40°F, atomized into a 25-mph air stream at 85°F. When the trees were removed from the spray tunnel after a 15-minute exposure, water-soaked areas were visible on many leaves. The trees were divided into 2 groups, one of which was placed on a greenhouse bench to dry. Trees of the second group were submerged in tap water to remove much of the surface deposit before being placed on the bench to dry. At the same time, unsprayed trees were dipped in a 65°F suspension of captan (2 lb. per 100 gal.) and divided into 2 groups, one of which was submerged in clean water before being set on the bench to dry. The experiment was conducted during a period of warm cloudy weather in late winter, when the older leaves were approaching mature size.

Within a week, typical captan injury developed in

TABLE 2.—*Injury to bean leaves by 2 per cent formulations of captan and different diluents in various ratios*

Captan-diluent ratio	Mean percentage injury[a] when diluent was as indicated		
	Kaolinite	Attapulgite	Talc
1:0.00	9.43	0.80	2.13
1:0.25	35.18	1.74	2.24
1:0.50	47.75	2.91	1.73
1:1.00	51.33	1.34[b]	4.13
1:2.00	60.02	0.27[b]	8.13
1:8.00	64.64	0[b]	12.73
0:8.00	0	0	0

[a] Mean of 48 spots, each on a separate leaf; 1 drop of each treatment was applied to each leaf.
[b] Excess flaking of dry deposit.

TABLE 3.—*Injury to bean leaves of various formulations of captan containing various amounts of a wetting agent (Igepal C A)*

Formulation[a]	Mean percentage injury[b] when concentration of Igepal was as indicated						
	% 0.0	% 0.12	% 0.25	% 0.50	% 1	% 2	% 4
Captan	10	20	28	33	42	42	70
Captan + koalinite	45	58	65	75	80	90	95
Kaolinite	0	0	2	10	20	40	55
Water	0	0	0	2	6	20	37

[a] Two per cent suspensions of all materials were used.
[b] Mean of 48 spots, each on a separate leaf; 1 drop of each treatment was applied to each leaf.

TABLE 4.—*Injury to bean leaves of captan-kaolinite formulations containing different concentrations of various inorganic compounds*

Concentration of compound ppm	Mean percentage injury* by formulations containing indicated compound				
	$Al_2(SO_4)_3$	$Fe_2(SO_4)_3$	$CaCO_3$	MgO	Na_2SO_4
0	57.9	57.9	57.9	57.9	57.9
1	60.3	62.9	58.6	51.9	55.9
10	57.9	55.0	50.1	38.1	26.8
100	61.8	66.3	40.6	26.4	40.2
1000	62.8	96.3	34.9	17.1	45.0
1000 (minus captan)	3.2	99.6	0.3	0.0	9.1

* Mean of 48 spots, each on a separate leaf; 1 drop of each treatment was applied to each leaf.

all sprayed trees. Very little injury was observed on the unwashed captan-dipped trees and no injury on the trees that were washed after the captan dip. This experiment indicated that spraying conditions resulting in stomatal penetration by captan greatly increased the phytotoxic response.

4) "*Safeners.*"—The inorganic compounds aluminum sulfate, sodium sulfate, ferric sulfate, calcium carbonate, and magnesium oxide were added in logarithmic series to a captan-kaolinite formulation to determine their effects on phytotoxicity. The concentrations of these compounds ranged from 1 to 1000 ppm (Table 4). Calcium carbonate and magnesium oxide had a "safening" effect on the captan formulation, whereas ferric sulfate *per se* was phytotoxic at the higher concentrations.

RESULTS OF STUDIES ON ENVIRONMENTAL FACTORS.—*Temperature.*—Since early observation of captan injury was noted particularly under conditions of high temperature, experiments were run in which bean plants treated with various captan formulations were placed in constant-temperature rooms ranging from 70° to 100°F. The over-all effect of temperature (Fig. 1) shows that phytotoxicity increased with increase in temperature, the critical range occurring between 85° and 100°F. Unlike the other formulations, the captan-kaolinite combination was injurious even at the lower temperature range. Figure 2 shows the straight-line relationship between duration of exposure and extent of injury when captan-kaolinite suspensions were applied to bean leaves at 90°F. When captan concentration was increased simultaneously with temperature, the resulting injury was more than additive.

Since the drops dried during the period of exposure

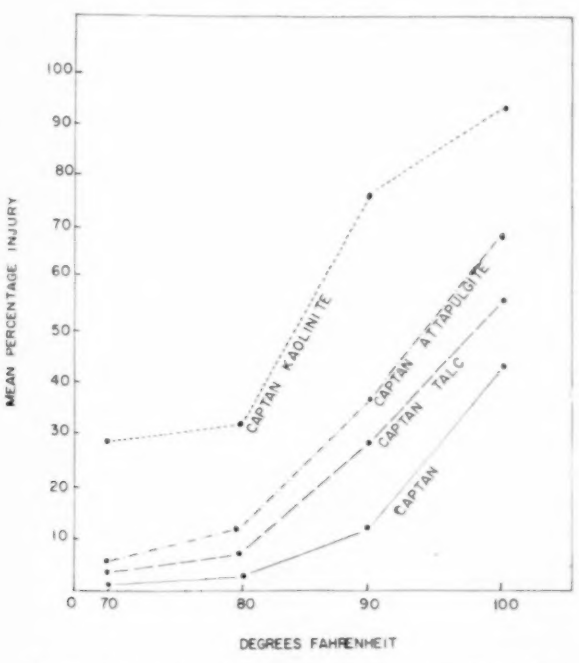

FIG. 1. Injury by a 2 per cent captan suspension to bean foliage at various temperatures.

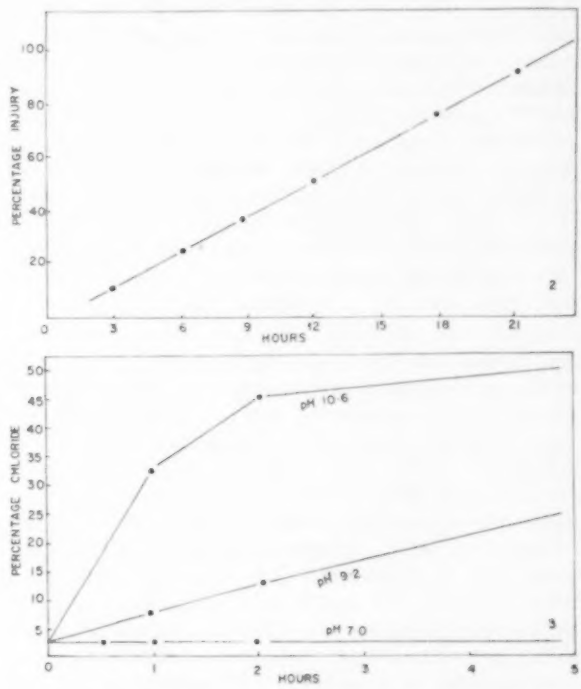

FIG. 2-3. Fig. 2. Injury by a 1:1 captan-kaolinite formulation (4% captan) to bean foliage exposed for various periods at 90°F.—Fig. 3. The percentage of chloride released from a 2 per cent captan suspension at various pH values after various periods of time at 68°F.

TABLE 5.—*Injury to bean leaves by formulations of captan, when droplets were dried or not dried before treated leaves were placed at various temperatures*

Formulation [a]	Condition when placed at indicated temperatures	Mean percentage injury [b] at indicated temperatures		
		80°F	90°F	100°F
Captan	Wet	0.0	0.0	0.0
Captan	Dry	0.0	2.5	6.7
Captan + kaolinite	Wet	25.0	46.3	55.8
Captan + kaolinite	Dry	49.2	92.9	89.6

[a] Two percent suspensions of all materials were used.
[b] Mean of 48 spots, each on a separate leaf; 1 drop of each treatment was applied to each leaf.

at 90°, no indication was given as to whether only wet leaves are injured by captan. To shed light on this point, drops of captan suspension were placed on bean foliage and allowed to dry at relatively low temperature and humidity to avoid injury during the drying period. Fresh drops of these suspensions also were placed on bean leaves; all plants were placed in constant temperature rooms at 80°, 90°, or 100°F.

Data in Table 5 also indicate that captan phytotoxicity increases with increase in temperature. It is also apparent that deposits that appear dry may be as injurious, if not more so, than drops that actually have free water.

Light.—Further experiments were conducted to determine the effect of light intensity on plant susceptibility to captan injury. Uniform bean plants were divided into 2 groups, one was placed under a greenhouse bench covered with cheesecloth and the other group was left on the bench exposed to the winter sun for 2 days before treatment. Two other groups of bean plants were held under similar light conditions for 2 days after captan treatment. All plants were subjected to captan treatment during the night. Two days later, injury data were recorded for all plants. Shading plants immediately before or after captan treatments increased foliage injury approximately one and one-half times over that of unshaded plants.

RESULTS OF CHEMICAL DECOMPOSITION STUDIES.—Before captan was first used in field studies by the senior author in 1946, its incompatibility with lime was ascertained. In these laboratory studies, it was learned that lime completely destroyed the fungicidal activity of the compound. Laboratory experiments conducted more recently have demonstrated that dry captan is relatively stable at ordinary temperatures; however, in the presence of heat or alkaline conditions, there is a rapid breakdown of the material with the formation of products, including HCl and H_2S; the rate depends upon the intensity of the factors. Chloride ion and hydrogen sulfide as well as pH were selected as criteria of captan decomposition in these tests. To study the rate of decomposition in an alkaline medium, 2 per cent slurries of technical captan were prepared in solutions buffered at pH 7, 9.2, and 10.6. Chloride determinations of these slurries held at room temperature were made over a 5-hour period. From these data, the ratio of chloride released during decomposition to that originally present in the captan molecule was calculated (Fig. 3).

Figure 3 shows that whereas little captan decomposition occurred at pH 7.0, the decomposition rate was very rapid at pH 10.6. When captan was dissolved in NaOH solution at pH 14, instantaneous decomposition resulted.

To determine the effect of temperature on captan decomposition, 2 per cent slurries of captan alone and in 1:1 ratios with kaolinite, attapulgite, and talc were maintained at various temperatures. Chloride determinations of the filtrates were made after 4 hours, 24 hours, and 1 week of exposure. Chloride data again were used to calculate the percentage of the chloride originally present in the captan molecule that was released during this period by decomposition (Fig. 4).

Figure 4 shows that chloride decomposition occurred rather slowly at temperatures below 110°F, but at 110° there was a very rapid rate of breakdown. Temperatures of such magnitude frequently occur on leaf surfaces under summer field conditions. There did not appear to be any great differences among the fillers in their effect on decomposition on the basis of chloride release.

Readings of resistance to an electric current also were made on filtrates from suspensions held at 90° and 110°F. When time and temperature were increased, the resistance of the solutions in ohms decreased (Table 6) or, conversely, conductivity increased, indicating greater ionization. This was taken as additional evidence of captan decomposition. Of the 3 fillers used, kaolinite appeared to have the greatest effect on captan breakdown.

The resulting pH of these solutions was also recorded. Acid production in the solutions also occurred rather slowly at temperatures below 110° (Fig. 5); at 110° there was a rapid decrease in pH, even with talc and attaclay, which remained fairly alkaline at the lower temperatures.

Finally, drops of these suspensions and their filtrates were placed on bean leaves and amount of injury recorded (Table 7). Both the suspension and filtrates

TABLE 6.—*The resistance to an electric current of suspensions of captan-filler formulations immediately after mixing and after 1 week at 2 temperatures*

Formulation [a]	Resistance in ohms		
	Immediately	After 1 week at temperature indicated	
		90°F	110°F
Captan	560	260	55
Captan-attapulgite	520	300	95
Captan-kaolinite	650	285	45
Captan-talc	560	290	135
Kaolinite	9,000	16,500	20,000
Attapulgite	2,250	1,600	1,800
Talc	5,700	3,300	3,850

[a] Two per cent suspensions of all materials were used.

produced the same type and amount of injury, indicating that the toxic factor was a soluble product of captan decomposition.

These data were compared with data on injury resulting from the application to bean leaves of drops of HCl and H_2SO_4 solutions in the same pH range (Table 8). Drops of HCl solution at pH 3.0 or lower also caused injury to peach and apple leaves.

Discussion.—These studies have partially explained the reasons for the injury observed on foliage treated

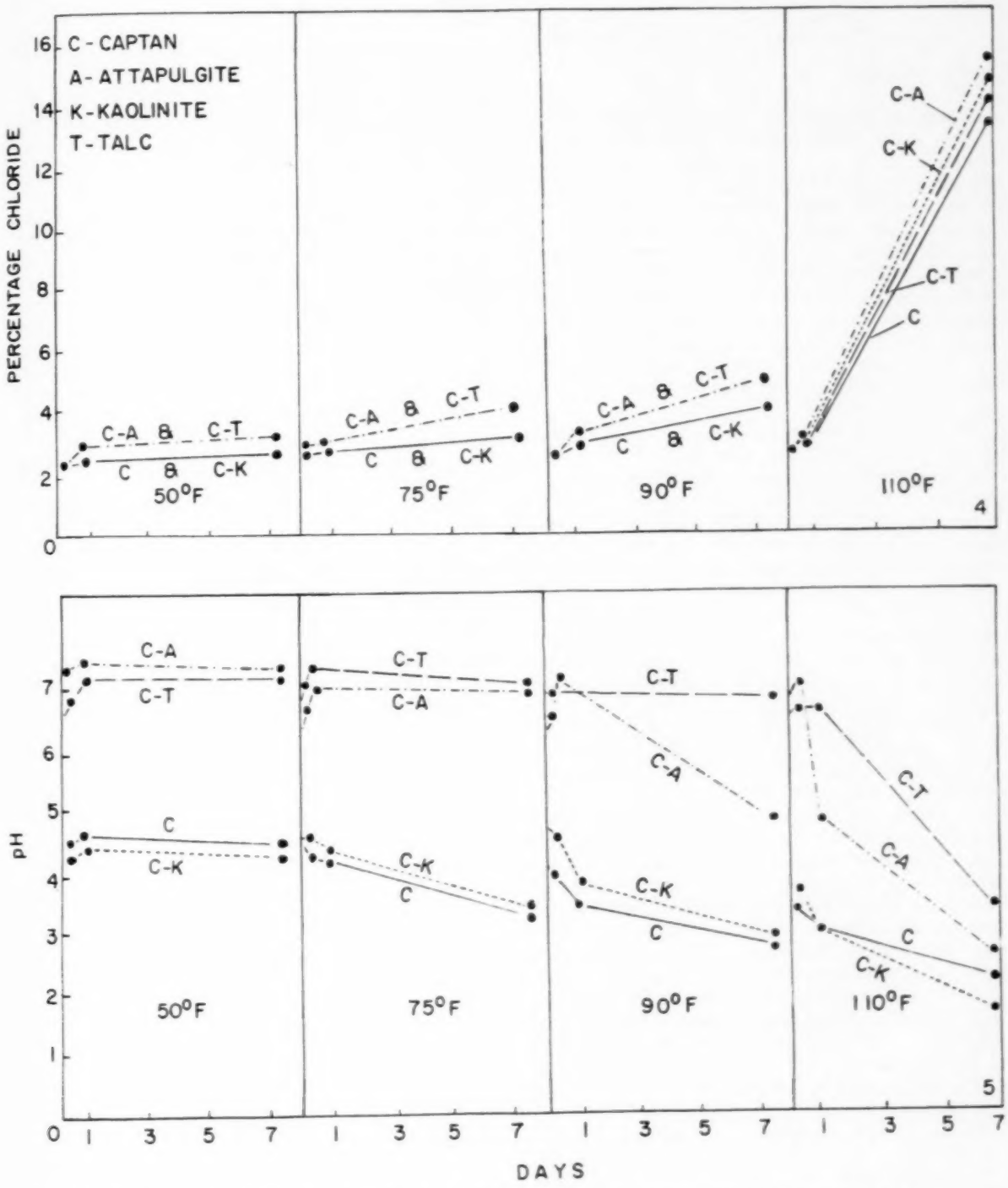

Fig. 4-5. The percentage of chloride released and pH of 1:1 captan-filler formulations (2% captan) at various temperatures after various periods of time.—Fig. 4. Percentage of chloride released.—Fig. 5. pH.

TABLE 7.—*The pH of captan-filler formulations and injury produced by them on bean leaves*

Formulation [a]	pH	Relative severity of injury [b]
Captan	2.0	++
Captan + kaolinite	1.8	+++
Captan + attapulgite	2.5	++
Captan + talc	4.9	—

[a] Two per cent suspensions of all materials were used.
[b] Based on 48 drops, each on a separate leaf; all treatments were applied to each leaf.

TABLE 8.—*The pH and amount of injury produced on bean leaves by various concentrations of HCl and H_2SO_4*

Acid	pH	Relative severity of injury [a]
HCl	1.5	++
HCl	2.0	++
HCl	2.5	+
HCl	2.6	+
HCl	2.8	Trace
HCl	3.0	—
H_2SO_4	1.5	++
H_2SO_4	2.0	++
H_2SO_4	2.5	Trace
H_2SO_4	3.0	—

[a] Based on 48 spots, each on a separate leaf; all treatments were applied to each leaf.

with captan formulations. Injury generally occurs during warm cloudy weather, when conditions are favorable for captan decomposition. Although there did not appear to be any differences among the fillers in their effect on captan decomposition when chloride production was taken as a criterion, results of resistance and pH measurements both indicated that kaolinite caused the greatest increase in ion concentrations in the filtrate, and talc the least with attapulgite intermediate. The failure of chloride tests to show this difference may be explained by the fact that although it has the lowest cation exchange capacity, kaolinite tends to retain the chloride ions produced during captan decomposition.

The differences among the fillers in their effect on captan decomposition and subsequent foliage injury may be explained on the basis of their physical characteristics. Kaolinite, with a fixed crystal lattice and a resulting low cation-exchange capacity, (3.8 milliequivalents per 100 g) lacks the power to absorb the hydrogen ions released during captan decomposition; this allows their concentration to increase rapidly to the point where injury occurs. Attapulgite has an expanding latticework that allows high reactivity with hydrogen ions (31.2 milliequivalents per 100 g exchange capacity); this results in a low hydrogen ion concentration and therefore little injury. Talc has an intermediate cation-exchange capacity of 10.8 and thus would be expected to be intermediate in phytotoxic effect; however, another factor to be considered is hydration of the various fillers. To investigate this point, mixtures of captan with fillers in 1:1 ratio were exposed at 77°F to relative humidities ranging from 51 to 93 per cent. Weighing measurements showed that at all humidities 3 times as much moisture was absorbed by attapulgite as by kaolinite. Talc showed little or no increase in weight due to moisture absorption. At the lower humidities, differences were not as great as at the higher humidities. Therefore, degree of hydration as well as reactivity must be considered in determining toxicity. Attapulgite, therefore, though causing a low hydrogen ion concentration, by its ability to absorb moisture prolongs the decomposition period and allows the pH to become lower eventually than that produced by talc.

Phytotoxicity, in addition to being influenced by physical and chemical factors operative in the fungicide film, is influenced by factors operative in the plant as well. This was indicated in the study of the influence of light on susceptibility of bean plants to such injury. In these studies, shading was found to increase phytotoxicity. Although the explanation for this response is not available, 2 facts are known that may influence susceptibility to injury. Under conditions of low light intensity, plants develop thin cuticles and cell fluids with increased hydrogen ion concentrations. Under such conditions, resistance to penetration into the leaf may be decreased, and injury from acids would be expected to be facilitated.

From these studies, it has been learned that captan may injure bean foliage in a few hours' time. It also has been learned that *in vitro* at experimental temperatures, captan decomposition sufficient to account for this injury requires several days' time. Perhaps this time difference can be reconciled by the fact that captan is soluble in the organic solvents that may occur in plant tissues. If solubility is increased slightly by such materials, the *in vitro* reactions discussed here would be greatly accelerated. Apple experiments have indicated that injury from captan resulted from stomatal penetration of the fungicide, hence the captan decomposition that is of most importance is that which occurs inside the leaf, where rates of decomposition cannot at present be measured. There is some evidence to indicate that phytotoxicity may result from captan within the plant cells. Laboratory tests in March, 1954, showed that amino acids and derivatives possessing a sulfhydryl group (cysteine and glutathione), inactivate captan as a fungicide. Reaction with sulfhydryl groups in the cell may provide an additional mechanism of toxic action, whereby captan is decomposed with the production of hydrogen and chloride ions and hydrogen sulfide.[9]

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[9] Daines, R. H. 1957. Foliage fungicides and combination sprays. Agricultural Chemicals 12: 32-34.

The Use of Uncoated Cellophane in Ultraviolet Irradiation of Microorganisms. VICTOR M. HELD AND ROBERT L. WALKER. Quartz or Corex vessels of the desired size and shape for irradiation studies are expensive and may be difficult to obtain. Pyrex beakers covered with uncoated cellophane are relatively cheap, effective culture vessels for exposing microorganisms to ultraviolet (UV) irradiation. The objective of such treatment might be to induce mutation or to study the behavior of certain microorganisms under specific portions of the UV spectrum.

If only coated cellophane is available, the coating can be removed by immersion in boiling water. The backing material separates and comes to the surface after about 5 minutes' boiling. The cellophane is then rinsed in distilled water and hung to dry. Cellophane treated in this manner becomes slightly wrinkled and there is a 10–30 per cent loss in its transmission at wavelengths below 290 mμ as compared to commercial uncoated cellophane.

If autoclaving is required, a 6-in. square of uncoated cellophane is tied over the mouth of each beaker. A cotton wick placed between the cellophane and the rim of the beaker, and extending below the fastening cord, prevents formation of a vacuum and bursting of the cellophane during cooling. Beakers of 250-ml capacity containing 50 ml of semisolid medium are most effective in our work, but other sizes can be used. The uncoated cellophane used in our experiments was generously provided by E. I. Dupont De Nemours and Company, Inc., Wilmington, Delaware. This material provides a tight, wrinkle-free covering.

The medium is seeded with spore suspensions injected through the cellophane with a hypodermic syringe. If the injection is made near the rim of the beaker, a small square of cellophane tape can be used to cover the resulting hole but we have not found this necessary. This technique has been in use for several months and a large number of cultures have been grown in this way without contamination.

With this technique, cultures can be given short exposures to UV or exposed to continuous UV irradiation of low intensity over periods up to several days. Since uncoated cellophane is highly permeable to water vapor, a high relative humidity should be provided in the incubation room if the cultures are to be maintained without transfer for over a week. Stray radiation may be excluded by covering the sides of the beakers with aluminum foil, paper, paint, etc.

The transmittance of uncoated cellophane as determined with a Beckman spectrophotometer, model DU, is shown in Figure 1. At the killing wavelength of 256 mμ, the uncoated cellophane still passes approximately 70 per cent of the incident energy.

Other materials tried had undesirable physical or transmission characteristics. Materials found unsatisfactory were coated cellophane, polyethylene, Saran, and Mylar. Uncoated cellophane, in addition to its good transmission qualities, was the only material that did not become coated with condensate, which would tend to reduce the transmission of UV.—Chemical Corps, Fort Detrick, Frederick, Maryland.

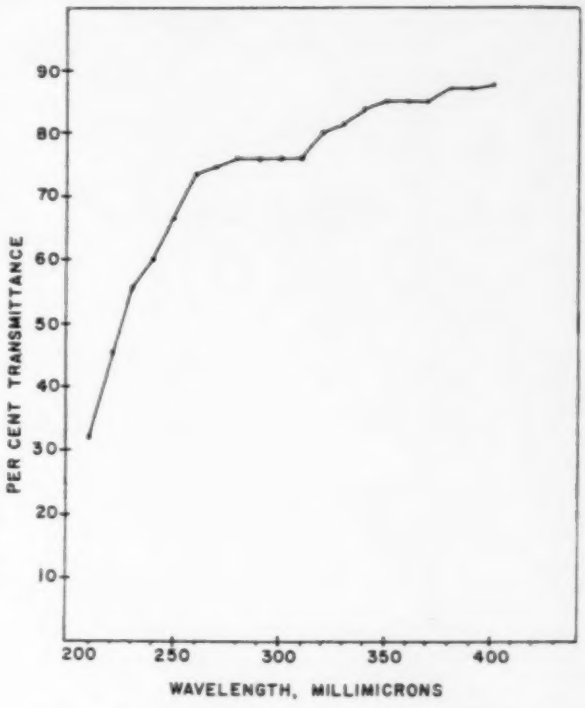

FIG. 1. Transmission characteristics of uncoated cellophane as measured with a Beckman spectrophotometer, model DU.

Local Lesions and Cross-Protection Studies with Bean Yellow Mosaic Virus[1] M. K. CORBETT.[2] The need for a satisfactory local-lesion host and a method for cross-protection studies with strains of bean yellow mosaic virus (BYMV), is evident from the numerous and conflicting reports concerning the identity of this virus. In attempts to find a satisfactory local-lesion host, over 50 plant species were tested with 2 strains of the virus. One strain was obtained by Hagedorn[3] from peas; the other strain was obtained by Diachun[4]

[1] Florida Agricultural Experiment Station Journal Series No. 609.

[2] Grateful acknowledgment is made to S. Diachun and D. J. Hagedorn for supplying the virus cultures.

[3] Hagedorn, D. J., and J. C. Walker. 1950. The relation of bean virus 2 to pea mosaic in Wisconsin. Phytopathology 40: 684-698.

[4] Diachun, S., and L. Henson. 1956. Symptom reaction of individual red clover plants to yellow bean mosaic virus. Phytopathology 46: 150-152.

from red clover. Plants usually were inoculated when the leaves had expanded to a satisfactory size but before flowering began. All inoculations were made by the gauze-pad method; leaves were dusted with Carborundum, inoculated, and then rinsed with tap water. Two species of plants, *Crotalaria spectabilis* Roth. and *Cassia tora* L., were found to react as local-lesion hosts. Because of leaf shape and plant size, *Cassia tora* proved unsatisfactory for quantitative work. Plants of *Crotalaria spectabilis* were easily grown by planting scarified seed in vermiculite and later transplanting the seedlings to pots of composted soil.

Plants of *C. spectabilis* developed 2 types of symptoms, depending upon the strain of virus used. The pea strain caused the production of small brownish-black local lesions, solid or ring type, 4–6 days after inoculation (Fig. 1,A); this was followed by a severe stem and tip necrosis.[5] Occasionally this systemic reaction proved lethal. The clover strain caused no visible symptoms on the inoculated leaves but eventually produced a conspicuous chlorotic mottle (Fig. 1,B).

Preliminary studies of dilution curves indicate that plants of *C. spectabilis* could be used satisfactorily for quantitative work with certain strains of bean yellow mosaic virus. Undiluted juice from *Pisum sativum* L. var. Alaska infected with the pea strain produced 200–300 lesions per leaf. Dilutions of 10^{-1} and 10^{-4} produced approximately 200 and 30 lesions per leaf, respectively. Occasionally 1 lesion per leaf was obtained at dilutions of 10^{-5}. The number of lesions obtained varied greatly with the source of the virus.

The facts that the clover strain produced mottle symptoms and the pea strain produced local lesions and systemic necrosis in *C. spectabilis* suggested that this host could be used to determine the immunological relationships of different isolates of the virus. Young plants inoculated with the clover strain developed a very strong mottle 20–30 days after inoculation. When these systemically infected plants were inoculated with the necrosis-producing pea strain as a challenge virus, good protection was evidenced. The established virus prevented systemic necrosis, but a few local lesions did form in the green areas of the mottled leaves. It is presumed that these occasional lesions occurred in spots that had not been invaded completely by the established strain of the virus. Attempts to recover viruses from the doubly inoculated plants invariably resulted in recovery of only the mottle-producing strain. When comparable healthy control plants were inoculated coincidently with the challenge virus, numerous local lesions and, eventually, systemic necrosis developed.

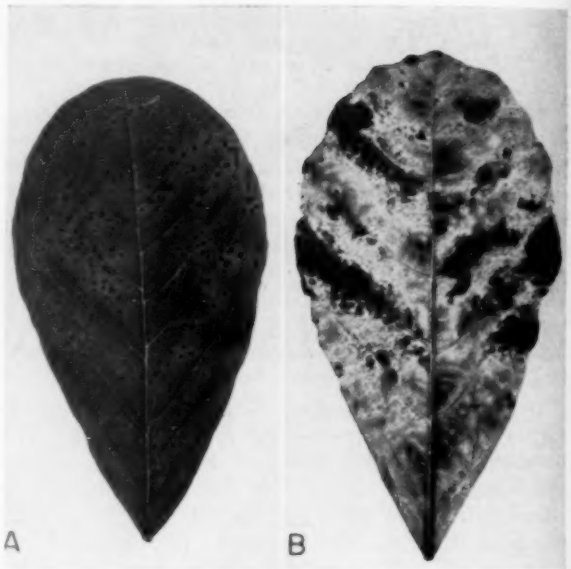

FIG. 1. Symptoms on leaves of *Crotalaria spectabilis* following inoculation A) with a necrosis-producing strain of bean yellow mosaic virus (approximate × 1/2) and B) with a mottle-producing strain (× 2/3).

C. spectabilis is also susceptible to infection by tobacco ringspot virus. Plants inoculated with tobacco ringspot virus developed large reddish local lesions and, eventually, systemic necrosis. Similar symptoms resulted when mottled leaves of this host previously infected by the clover strain of BYMV were inoculated with tobacco ringspot virus; no evidence of immunological relationship between the 2 viruses was obtained. This lack of protection between the clover strain and a distinct virus indicates that the protective action between strains of BYMV is more than a non-specific interference reaction in plants of *C. spectabilis*.

The use of *C. spectabilis* and an appropriate strain of virus has facilitated identification of several strains of BYMV that have been isolated from leguminous crops.—Plant Pathology Department, Agricultural Experiment Station, Gainesville, Florida.

[5] Hungerford, C. W., and I. G. Hillyer. 1954. Yellow bean mosaic in Idaho. Plant Disease Reptr. 38: 621-627.